*THE AMERICAN JOURNAL*
*of*
# HUMAN GENETICS

# THE AMERICAN JOURNAL of HUMAN GENETICS

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Volume 10
1958

Published Quarterly by
AMERICAN SOCIETY OF HUMAN GENETICS

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Baltimore 2, Maryland

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SIR ARCHIBALD E. GARROD, K.R.C.P., F.R.S.

*The American Journal of Human Genetics*

*Vol. 10 No. 1* *March 1958*

# Some Reminiscences of Sir Archibald E. Garrod, K.C.M.G., F.R.C.P., F.R.S.

TO ALL MEN trained at St. Bartholomew's Hospital in the second decade of this century, Sir Archibald Garrod was well known as a very respected member of the staff. My main contact with him was when I became Professor of Biochemistry in Oxford from 1923 to 1928, at the time when Garrod was Regius Professor of Medicine, having succeeded Sir William Osler in 1921.

In 1923–24 negotiations with the Rockefeller Foundation were in progress and Sir Archibald had much to do with the large grant which came from them to Biochemistry for their building, teaching and research in 1924, a grant arranged also to give extra space for Sir Charles Sherrington to expand his work. During all these early days in Oxford Garrod gave valuable and unselfish help.

During World War I, Garrod became Consultant Physician to the forces which took him to Malta where his advice was very greatly valued, and in 1919 he was created K.C.M.G. for his services. It was a tragedy that he lost his three sons during 1916 to 1918.

Garrod must have had his biochemical and clinical interest in his chromosomes because his father Sir Alfred Garrod, well known for his work on rheumatism and gout, published one of the first micro-methods for blood, this being the estimation of uric acid in the circulation of gouty patients. The writer well remembers Archibald Garrod referring to his boyish remembrances of the watch glasses with threads on them on his father's consulting room mantelpiece on which the uric acid was selectively adsorbed.

Garrod had a usual British education at the school of Marlborough and at Christchurch, Oxford. It may be that his latent biochemical interests were stimulated by the fact that he was forced to study chemistry at Oxford; at that time there was no special course for medicine. This may have had some influence too in deciding his subsequent research interest, because, along with the busy life of a physician and various appointments to several hospitals in London, he retained all his life a great interest in problems of chemical pathology, and he used to tell me how he often went down to Guy's to discuss his work actively with Gowland Hopkins.

After some research on urochrome and uroerythrin and later urobilin, in 1897 he came into touch with a case of alcaptonuria and it was this which led to his specialization in what he called "Inborn errors of metabolism," a very happy phrase.

The work to which this introduction is a preface indicates how important was his observation and how right he was in his interpretations. Quite early he held firmly the theory that it was a missing enzyme which was responsible for those abnormalities at a time when many of us found it difficult to take such a view. Several of us enjoyed the one or two lectures in this field which he used to give each year to the Final Honour Class in Physiology.

The book on "Inborn errors of metabolism" of which the second edition is perhaps the best known, was published in 1923; it is outstanding amongst scientific publications in its beautiful use of the English language. Garrod himself always said that as he grew older he found that he became more fastidious. I can recall one instance of this when I took, as I thought, a final draft of my Inaugural lecture in Oxford to Archibald Garrod. He read this through and said that he thought it would cover things well, but suggested that I should now take it away to put a "curl in the English"! In conversation and in lectures, he was particular and happy in the turn of his phrases. His intense interest in the problem of porphyrins can perhaps best be indicated by the fact that he would ring me up often nightly, when new papers on the porphyrins were sent to him by Hans Fischer.

A small book which is not so well known entitled "The Inborn factors in disease" was published in 1931, and was based upon the Huxley lecture on 'Diathesis' delivered at the Charing Cross Hospital in 1927. It contains many valuable thoughts, and gives a reference to a stimulus Garrod himself received from a Rectorical Address given in Prague in 1896 by Huppert who "agreed that chemical differences in the constituents of the germ cells must determine the attributes of the organizations developed from them."

Garrod was a most charming man with quiet and unassuming dignity and above all, wisdom. After retiring from the Chair in Oxford, he went to Cambridge to be near his daughter Professor Garrod, a Fellow of Newnham College, well known for her work on prehistoric archaeology. He often stated that he thought it strange that a Regius Professor of Medicine in Oxford should retire to Cambridge!

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# Sir Archibald Garrod's "Inborn Errors of Metabolism"

## I. Cystinuria

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"It is an old experience that Nature often grants us through her errors unexpected insights into her secrets, which are otherwise a closed domain." *Über Cystinurie.* A. Loewy and C. Neuberg, 1904 (54).

### INTRODUCTION

THE OCCURRENCE in certain individuals of renal or bladder stones composed of pure cystine has attracted attention since the early nineteenth century. The specific chemical nature of the anomaly, and its frequent familial incidence, guaranteed its interest to different investigators, just as the dramatic symptoms of renal colic often seen with the condition compelled the interest of physicians. It has a further claim on our interest as one of the bases of a great theoretical advance. It was one of the four diseases singled out by Sir Archibald E. Garrod in 1908 as the "inborn errors of metabolism." This concept grew out of considerations of all facets of the disease, both scientific and clinical, and the same integrated consideration has been attempted in this history of the study of this disease written fifty years later.

Cystinuria is an hereditary anomaly of renal function, with defective tubular reabsorption of cystine, lysine, arginine and ornithine. These amino acids, two of them essential to the body, are excreted in the urine in abnormal amounts throughout life. Only the excretion of the least soluble one, cystine, was recognized until recent times, and both the name and the clinical importance of the condition is entirely referable to this one amino acid. The only clinical consequence appears to be the frequent formation of urinary calculi composed of almost pure cystine. Most individuals homozygous for the cystinuria gene sooner or later form such stones, and the stones tend to recur. The sequelae may well lead to eventual renal insufficiency and death. Some of the individuals heterozygous for the cystinuria gene also excrete more than the normal amount of cystine, which can be detected by chemical tests such as the cyanide-nitroprusside reaction, but the amount here is not great and these individuals only very rarely form cystine stones. The incidence of chemical cystinuria is nevertheless much greater than the incidence of stone-forming cystinuria, since heterozygotes are vastly more common than homozygotes, and not all homozygotes have stones at a given time.

The separate identification of heterozygotes and homozygotes is essential for extending our knowledge of this disease, and this requires more than the qualitative

* This laboratory is supported by U. S. Public Health Service Grant A567 and by U. S. Atomic Energy Commission Contract No. AT (30-1)-901 with the New England Deaconess Hospital.

recognition of cystine excretion. A quantitative determination of cystine is also needed for the proper treatment of those subject to clinically important complications. It is now reasonable to believe that cases diagnosed as homozygotes before calculus formation has occurred can be successfully managed so that all complications will be prevented. However, this successful treatment depends upon the full understanding of the disease mechanism.

The possibilities of treatment and of further research can best be appreciated in the perspective of the studies that have been done in the past. Not only are there many nearly forgotten things that need not be rediscovered, but there are also errors commonly accepted as fact. The clinical literature, in particular, has perpetuated a number of beliefs about cystinuria that were long ago shown to be false. The history of the investigations of cystinuria also provides an unusual opportunity to trace the laborious development of human understanding about a disease. Particularly is this so, since the history can now be written from the vantage point of a reasonably complete understanding. Our knowledge grew by the successive solution of limited problems: first, the clinical definition of the disease; the chemical nature of cystine and its role in metabolism; then the relation of heredity to metabolic events; and finally the specific biochemistry of renal physiology. But this stepwise progress was not intentional on the part of the successive investigators. In each generation the investigators were concerned with the integration of the whole problem, were plagued by what they did not yet understand, and were often uncertain even about what was definitely established. These human frailties and errors are not without interest and instructional value, but the epic quality of the investigations of cystinuria is most apparent in relation to the different concepts of mechanism which, one after the other, flowered and faded until one, for the present at least, has provided an adequate basis of understanding for this disease and related ones.

## I. EARLY STUDIES

The opportunity to investigate this relatively rare disease occurred only sporadically and was not always given to those best prepared to advance our knowledge. Even more importantly, the cystine with which such studies began was provided by the patients themselves, upon whom the supply of this essential material was dependent for nearly 100 years (in 1904 (54) and even in 1936 (52) cystine metabolism was tested by giving to the individual cystine from a stone which he had formed). For these reasons Niemann could well bewail in 1876 that "our knowledge of cystinuria has indeed developed unusually slowly and by small degrees" (64). Through these limitations of clinical and chemical materials the knowldege of cystinuria was slowly lifted by its bootstraps.

Cystine, and cystine stones, were recognized before the patients with cystinuria. Wollaston, who had thirteen years earlier described five different types of urinary calculi, added a sixth in a report to the Royal Society of London in 1810, "On Cystic Oxide, a New Species of Urinary Calculus" (86). Two examples of bladder stones had come into his hands, one from a collection at Guy's Hospital ("No. 46—according to the present arrangements—which, it is to be hoped, will not be altered") and

one from a physician. "It had been taken from his brother when he was five years old." Wollaston had considered the substance to be an oxide because of its tendency to unite with both acids and alkalies. From its occurrence in the bladder, he gave it the name "*cystic oxide*, which will serve to distinguish it from other calculi; and as this is unlike any other term at present employed in chemistry, it is to be hoped that it will not be thought to require any alteration."

But the name became a focus in the first debates about the new disease. The next few cases also had bladder stones, perhaps not simply because "Was mann weiss, sieht mann," as Goethe observed about that time. Stones do develop in the bladder "remarkably often" in cystinuria (70), especially in young people. It was assumed that cystine stones were therefore formed by the bladder, until Marcet in 1818 found three patients (two at autopsy) with renal stones of cystine. He suggested that *nephritic oxide* was a better name. "Venables suggested *nephrine*, but Civiale objected to this term as unphysiologic. Instead he suggested *scorodosmine*, alluding to the odor of garlic given off by the substance when heated before the blowpipe" (70). Chemistry had its say through Berzelius, who observed that whatever its structure, the substance was not an oxide. He suggested the name *cystin*. "Of this new name, which has since been universally adopted, Civiale wrote, in 1838, that although it corrected an error of chemistry it perpetuated an error of physiology, for cystine is excreted by the kidneys and does not have its origin in the bladder" (Garrod, Lecture III (32)).

The renal origin of cystine was established when Prout in 1820, and Strohmeyer in 1824 (cited in 70), recognized the same hexagonal platelets of cystine in urinary sediment, which Wollaston had formed from dissolved stones. The identification of these crystals through a microscope has remained until recent times the primary means of diagnosis of cystinuria. The collection and weighing of these crystals was first done, according to Renander (70), in 1855 by Toel, who found a cystine excretion of 1.33 to 1.50 grams per day in a cystinuric. This procedure also became the basic method for the study of cystine metabolism until it was displaced after 1900 by Folin's urinary sulfur fractionations.

## II. CONTRIBUTION OF NIEMANN

In the study of the literature of a relatively rare disease, where every "Case Report" is "With a Review of the Literature," the superficial and the fragmentary bury the rare work of scholarship. Also, errors tend to be perpetuated by plagiarism. Two works on cystinuria stand out among less than a dozen authoritative reviews, one in 1876 by Niemann, and one, almost never quoted, by the Swedish radiologist, Renander, published in 1941. References not otherwise identified here can be found in the latter work, which cites correctly and fully, for the most part, the literature on cystinuria to that time. This literature was first given form by Niemann.

The slowly accumulating information from sporadic cases of cystinuria was harvested and winnowed in 1876, when the writing of Niemann's medical Inaugural Dissertation at Göttingen happily coincided with the referral of a case of cystinuria to his professor (Ebstein). From what began as a case report by a young medical man willing to undertake some chemical investigations on his patient grew the

first definition of this obscure medical condition. Stimulated by the fact that the latest review, published two years earlier, had listed only fourteen cases of cystinuria, Niemann tabulated in chronological order and discussed, in addition to his own patient, fifty-two cases which he found in the medical literature. He consulted the original references for most cases. Subsequent reviews and research followed the pattern he set in this first authoritative discussion of the disease.

*Diagnostic Problems:* Nearly all of the cases in Niemann's collection had been diagnosed on the basis of urinary stones of cystine, although several had been recognized by cystine crystalluria. He provided careful drawings and crystallographic studies of these transparent, shiny, hexagonal leaflets seen with a microscope in the sediment of urine which had been allowed to stand for a few hours. Although he called the recently described nitroprusside reaction, which gave a violet color with cystine, an "elegant" test, the identification of cystine rested primarily upon Wollaston's criteria: the solubility of the crystals in ammonia and the insolubility in acetic acid, and, when strong HCl was added to the dry crystals under the microscope, the rapid growth of stellate clusters of delicate prisms consisting of the water soluble cystine hydrochloride. Chemical analysis depended largely upon the "sulfur reaction," the formation of a precipitate of lead sulfide when urine or a cystine solution were heated with KOH in the presence of lead acetate. He recognized that cystine could be present in the urine, as indicated by the lead sulfide precipitate, in abnormal amounts without the presence of stone or crystals. The brother of his patient had such abnormal amounts of dissolved cystine. However, Niemann did not make a definite diagnosis of cystinuria on this basis.

By his criteria, a probable diagnosis of cystinuria, suggested by a stone or crystals, was to be confirmed by the identification of cystine through the strong sulfur reaction given by the solid material. Additional confirmatory evidence which he mentioned was the smell of hydrogen sulfide, occurring when the urine was allowed to stand for many days, and the development of a particularly foul smell in the urine in hot weather or when admixed with rotting urine. The latter was no doubt due to the cadaverine and putrescine formed by bacterial action on the other amino acids now known to be present in these urines in abnormal amounts. Since the methods for detecting cystine depended upon supersaturated solutions from which it would precipitate, it is not surprising that he was cautious about making or excluding the diagnosis of cystinuria. He noted that cystine crystals were sometimes absent from the urine of individuals with a known cystine stone; that cystine crystalluria was sometimes present without stone; and in several cases, that stones of other chemical constitution were found in known cystinurics. The latter are now known to develop with the aid of the urinary infections set up by the original cystine stones. With the diagnostic precautions used by Niemann, it is evident that patients with cystinuria might be missed, but few who were not cystinurics would be diagnosed as such.

*Patterns of Occurrence:* Both males and females were affected with cystinuria, the males predominating. Thirty-eight males and fourteen females, with one patient of unstated sex, made up Niemann's collection of fifty-three patients. A similar predominance of males, though slightly less marked, has characterized all subse-

quent collections of cases (47, 50, 59). Niemann attributed this difference to a natural reluctance of women to see a doctor, and to the greater ease with which small stones were passed through the female urethra, with the result that fewer women than men were forced to seek medical help. Whether or not this simple explanation gives the correct reason, women were once upon a time more reluctant than men to go to a doctor. Now this reluctance is vanishing, and with it is disappearing the sex difference in incidence of cystine calculi. No significant sex difference occurred in a group of cases diagnosed by chemical examination (40).

Niemann believed that cystinuria might well be present from birth. Although it has not even yet been observed in a new-born child, the youngest patient seen at that time was two years old. Since then a cystine stone has been passed spontaneously by a child of nine months (Case VI (62)). The ages of the patients were known to Niemann in thirty-six cases, which revealed that there were similar frequencies of stone formation in the successive decades of life (Age $< 10$ yrs., 5; 10–20 yrs., 6; 20–30 yrs., 12; 30–40 yrs., 7; 40–50 yrs., 6), with no stones then reported to have developed after age 50. Renander tactfully mentioned that Niemann overlooked two cases, aged 61 and 73 (70). Kretschmer found a similar distribution with age in 107 cases, 73 per cent of which developed by age 40 (47). In one patient stone formation occurred at age 42, and recurred at age 81 (81). The oldest case reported was a woman of 87 (57). Because no particular age was singled out, and since actual diagnosis of stone was often preceded by many years of symptoms suggestive of urinary lithiasis, Niemann believed that the underlying cystinuria was lifelong, with stone development occurring sooner or later in most individuals with this condition.

Niemann was led to the latter conclusion, that most cystinurics ultimately formed stones, because persons with only cystine crystalluria were even rarer than those with stones. The incidence of stones in an unbiased series of cases, i.e. one selected chemically and not on the basis of clinical symptoms of stones, was recently estimated to be well above 50 per cent of homozygous cystinurics (22). Niemann mentioned the possibility that abnormal amounts of cystine in the urine detectable by chemical methods might be more common than generally thought. This H. B. Lewis demonstrated fifty years later to be true (50). It was impossible to make an estimate of the absolute frequency in the population of cystinuria characterized by cystine stone or crystalluria, and Nieman pleaded for the study of urine samples from large numbers of individuals to supply this data. The relative rarity of the condition was clearly attested by the number of cystine stones among all urinary calculi found in clinical practice or in museum collections. He cited the finding of three cystine stones among 649 urinary stones from the Hunter Museum, and the experience of three clinics treating urinary calculi with reported incidences of cystine stones in 1 in 300 patients, 6 in 1100 female patients and 4 in 105 male patients. This incidence of cystine stones, making up approximately 1 per cent of all urinary calculi, has been generally confirmed by all subsequent surveys (63, 78), of which Mörner's is the most complete (58).

Niemann mentioned that observers had been impressed with the familial disposition of cystinuria. The fourth and fifth cases, described by Marcet in 1818, were

two brothers about thirty years old. Four other instances in which two siblings were affected had been reported, and in one of these instances, two sisters with cystine stones, the mother had also excreted abnormal amounts of cystine but did not have stones or crystalluria. It was quite unclear to Niemann at this time, before the rediscovery of Mendel's work, why a number of these cases showed this striking familial tendency, and yet surveys of the families of other patients failed to reveal any additional cases. Of course, most of the individuals studied in such surveys belonged to earlier or later generations than the propositus, and affected cases of a recessive condition would not usually be expected except among siblings. It is interesting to note that the brother of Niemann's patient, who might well have had the same genotype, excreted abnormal amounts of cystine by chemical test, while the mother and maternal uncle of the patient were normal in this regard. A passage in Niemann's paper states that despite the care with which a case was studied ". . . in unserer Kenntniss über die *genetischen* Beziehungen der Cystinurie nicht weiter gebracht." This curious anticipation of a word soon to be used in another sense must refer simply to the failure to learn more about the origin or pathogenesis (genetischen Beziehungen) of the disease, since nothing was then known about the genetics of this or any other condition.

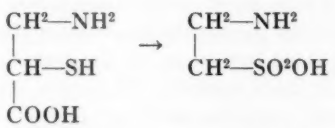
*Possible Causes:* His large collection of cases put Niemann on firm ground to evaluate the alleged "constitutional" or environmental causes of the disease, which were usually based on coincidental findings from single causes. Since five patients were less than ten years old, he dismissed all possibilities of an association with an occupation. Six patients were of high social standing, and for this reason he dismissed the suggestive influences of poor housing and coarse nutrition on the development of the disease. He admitted that "scrofula, chlorosis, and anemia" were sometimes associated with cystinuria, but considered them incidental to, or results of, the complications of cystinuria, rather than predisposing causes. The cases of the two v. Planta brothers reported by Civiale confirmed his view that one need not be sickly to have cystinuria: they were men "of the strongest nature, without any apparent constitutional disturbances, and in the most fortunate of circumstances, yet they had the most marked degree of cystinuria." An alleged association with rheumatism had been based on a theory that rheumatism was caused by cystine crystals in nerves and blood vessels, and in chronic cases the crystals appeared also in the urine. This he mentioned merely "as a curiosity," since most cystinurics were without a trace of rheumatism. Yet statements that there may be this same association could be found in medical writings more than fifty years later (50)! Niemann exhibited a similar restraint about incidental findings in the study of his own case. This eighteen year old student had an intention tremor of the upper extremities. Niemann did not wish to attribute this finding to the coexistent cystinuria, but suggested that it might be a consequence of the fact that "the boy drank more beer than his parents knew about." Niemann was in a position to estimate accurately the intake from his measurements of the daily urinary output.

The most scathing remarks were reserved for the then current hypothesis that Marowsky had developed in 1868 about cystinuria. This hypothesis about the disease mechanism emerged after the observation of a single case of cystinuria, in a patient

who also had chronic acholia and liver disease (56). Taurine, excreted in the bile as taurocholic acid, and cystine were both known to contain sulfur. Marowsky suggested that cystinuria was caused by liver disease. He believed that in his jaundiced case of cystinuria the usual taurine-secreting activity of the liver was suppressed, and replaced by "vicarious secretion of the sulfur-containing taurine through the kidney in the form of cystine."

Niemann said of Marowsky, "I will only remark that the cystine excretion in his case was not continuous, while the acholia and disturbed liver function were continuous. One may demand at least a continuous 'vicarious activity' of the kidney if this view is to be represented as plausible." It seemed unlikely to Niemann that cystinuria could be the result of underlying liver disease, with conversion of taurine to urinary cystine, without the appearance in the urine of other parts of the bile acids. To this point he adds the observations that neither taurine nor cystine could be found in the urine of other patients with jaundice. Lewis later cited analyses showing no deficiency of taurocholic acid in the bile of cystinurics (50).

Niemann nevertheless revamped Marowsky's hypothesis into the form later to be known as an "arrest of metabolism," a concept from which Garrod developed his fertile idea of the "inborn errors of metabolism." Instead of taurine being converted to cystine, as Marowsky implied, the formulas of the substances, as then known, indicated to Niemann that taurine was the "natural oxidation product of cystine":




Cystin Taurin

(According to Niemann, 1876)

"Should one reason from these pure chemical considerations to the situation in the human organism, one would think that in certain cases, from unknown causes, cystine was not oxidized to taurine and therefore appeared in the urine." Niemann had to point out, however, that plausible as this scheme was from a theoretical standpoint, the current knowledge of animal metabolism provided no support for it. He believed that wider investigations of other separate substances in the urine should be undertaken. His own measurements disclosed a low sulfate excretion in cystinuria. Such investigations, in time, would reveal the other amino acids present in abnormal amounts in cystinuria. Niemann failed to add the now obvious suggestion that a failure of cystine oxidation would cause the low urinary sulfate, but the pathway of cystine oxidation through taurine to sulfate was not yet known.

*Treatment:* No treatment was used on Niemann's case: "All means attempted up to now have proved useless." He thought treatment would continue to be unsuccessful until based on an understanding of the origin of cystine in the organism. On this subject he was ironic: "In the beginning, when cystine was known only to exist in bladder stones, it was believed to be formed by the bladder, and when cystine

was later found in the kidney, that was believed to be the site of formation; and Scherer's discovery of cystine, though only once and in the liver of an alcoholic, suggested the liver as the possible place of origin of the cystine." He reviewed earlier attempts at treatment, which included seltzer water to increase the urine volume and minimize precipitation. He suggested, apparently for the first time, that alkalinization of the urine might dissolve the cystine (Magendie is credited with the same suggestion, plus restriction of protein, in 1828 (70). But on one occasion his patient excreted an alkaline urine containing cystine crystals. This observation, plus the fear of precipitating phosphate with alkali and so enlarging the stones already present, kept him from trying alkali therapy. Only surgical treatment was to be offered when necessary, and that sometimes tardily by modern standards, so it was fortunate that many of the patients spontaneously passed their stones. In the group of those who were not candidates for the surgery of the day he included "a woman of 50 years who passed 13 cystine stones through a fistulous opening between the symphysis and the navel."

Niemann's success in defining cystinuria, achieved largely by the use of logic to divest the known cases of their adventitious trappings, can be measured by comparing his conclusions with the definition of cystinuria written in 1955 by Dent and Senior (22): "It is a condition presumed to be present from birth and characterized by the excretion in the urine of large quantities of cystine (in the adult, about 1 g. in 24 hours), lysine, arginine and ornithine. The condition is often present in the patient's siblings and may result in the formation of stones composed almost entirely of cystine. Apart from the possibility of kidney damage and the other complications resulting from stone formation, the patients enjoy good health and are clinically indistinguishable from normal." Niemann's definition agrees with this modern one in every detail, with the exception only of the abnormal amounts of lysine, arginine and ornithine in the urine of these patients.

### III. STATISTICS AND CLINICAL CONFUSION

Niemann's review altered the character of the clinical reports on cystinuria which appeared later. Little could be added of clinical importance, except validation of his definition of the disease, until additional understanding was available. Clinical interests were contented by the accumulation of statistics. Ebstein, Niemann's professor, did him the honor of republishing his count eight years later and adding ten cases. One of the best of the early clinical articles was that of Simon (74), who reprinted Niemann's chronological list of fifty-two reported cases and brought it up to 1900, making a total of 103 cases, plus four of his own and one from the Norwegian literature added to the paper when in proof. One of his cases was a Negro, the only one reported to have cystinuria. In general, Simon repeated Niemann's arguments about the nature of the disease. Though without direct reference to them, the cases collected by Niemann and Simon were repeated in the collection made by Kretschmer in 1916 (47). This also contained 107 cases, though more had been reported in the interim since Simon's paper. In fact, three more reviews, of 114 cases in 1904 (Wasserthal), 153 in 1907 (v. Hoffmann) and 164 in 1912 (Link) had been published before Kretschmer's article. In the ten-year period between

1921 and 1932 Lewis counted seventy-one cases reported (50). Morrison listed seventy-five cases studied radiographically between 1920 and 1940 (62). Sum-Schick counted 180 by 1929 (78). Renander's estimate that published cases by 1940 "only slightly exceeds 200" was conservative.

Until 1932 case finding depended solely on the identification of cystine stones or crystals in urines "of patients presenting symptoms of renal colic or calculi of the genito-urinary tract. Cystinuria uncomplicated by calculus formation has been detected almost entirely by examination of the urine of relatives of patients who have been compelled to seek surgical aid for the removal of calculi" (47). But the number of cases seen depended on the interest in the disease. For example, the first case was seen in Sweden in 1870 and the second only in 1901. Only six were seen from 1901 to 1920, and these were reported by Mörner. In the five years following this, galvanized by Mörner's continued interest, sixteen additional cases were observed, four of whom did not have stones (59). These latter were discovered in surveys of the families of known cases and were diagnosed by the presence of cystine crystalluria. The diagnostic criteria had not changed. The stones might resemble triple phosphate stones, as mentioned by Wollaston, and even be admixed with mineral deposits; if not investigated more thoroughly they would not be properly identified. There were consequently serious limitations to the estimates of the absolute incidence of cystinuria. But the surveys of large numbers of urine samples for cystine crystalluria pleaded for by Niemann had uncovered one case in 15,000 (74), one case in 20,000 (Primavera, cited in (34)) and four cases in approximately 35,000 urine specimens (75). Since the crystalluria was known to be intermittent in closely observed patients, the true incidence of cystinuria was undoubtedly higher, but it was not imagined how much higher chemical methods would show it to be. For some time cystinuria was regarded as a rare condition, fortunately since Garrod used rarity as a criterion for an inborn error of metabolism.

Stone formation is indeed a rare occurrence. Something like a complete ascertainment of the incidence in the generation of the Swedish population living between 1901 and 1936 was assembled by Mörner. He had found a total of thirty-six cases by 1936. At that time Renander surveyed all the hospitals in the country and succeeded in finding only one additional case (70). This gives an incidence of stone-forming cystinurics in a population of six million of roughly 1:200,000.

The usual interest was shown in the unusual case. Southam (76) removed a cystine stone from a woman who, fourteen years post-operatively, still excreted cystine. The mother of this patient had been operated on twenty-four years earlier by Southam's father, also for cystine stone, and she continued to excrete cystine for eight years. The chronicity of the disease was further documented by a metabolic study in 1923 (55) of a patient studied seventeen years earlier by Alsberg and Folin (3). The metabolism of the patient had not altered. But the myth persisted in clinical circles that removal of a cystine stone might cure the cystinuria.

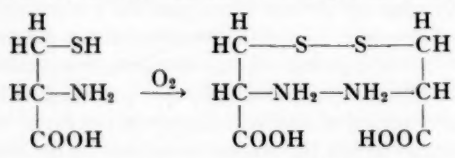
A dignified rivalry to report the largest stone can be discerned in the clinical literature, but chaos governed the contest. The participants, who frequently confused weights in *grams* with *grains* (1 g., gm. or gr.[am] = 15.4 gr.[ains]), always to enlarge a stone about ten-fold (see 70 for references), are fortunately no longer our

family physicians. The record, attested by an independent weighing twenty-seven years later when the stone reposed in the Museum of the Royal College of Surgeons in England, was long held by a vesical calculus weighing 68.04 g. and described by Harrison in 1879. Link found a stone of 86 g. in 1912, but all records toppled when Ancherson in 1914 removed from the bladder of a Norwegian man a cystine stone weighing 102.5 g.

A most notable confusion about the clinical nature of cystinuria was introduced by Abderhalden's description in 1903 of another disease of cystine metabolism. Although Abderhalden called the new disease *familial cystine diathesis* (1), not *cystinurie*, the majority of medical writers up to the present time have considered it to be a manifestation of the same hereditary disturbance as cystinuria. After the next cases were described, in 1926 by Lignac-Leiden, it was also called cystinosis, cystine storage disease and Lignac-de Toni-Fanconi syndrome. The first patient was seen only after death had occurred at the age of twenty-one months following symptoms of inanition. The internal organs were spotted with crystalline deposits of cystine. Abderhalden demonstrated abnormal amounts of cystine in urine of two siblings, the father (9.2 mg/100 ml) and the grandfather (14.0 mg/100 ml) of his patient. A reason for additional confusion was that Lignac's first case had ureteral and renal cystine stones (53), a unique finding in any condition except cystinuria. The typical findings of cystinosis, all of which distinguish it from cystinuria, are the retarded growth and development, the rickets and osteomalacia, the low serum phosphorus, the glucosuria, the generalized aminoaciduria of ten or more amino acids among which cystine is usually not prominent, the very rare formation of cystine stones, the presence of cystine crystals in the tissues of children but not adults with the disease (25), and its frequently fatal termination.

## IV. THE METABOLIC MAZE

A more precise definition of cystinuria was dependent upon knowledge of the chemical nature of cystine and of its metabolism. It would seem that both these aspects were well on the way to solution by 1905. In 1900 Simon (74) had complained that little was known of the mode of formation of cystine in the body because it had not been synthesized and investigators were dependent upon the supply of cystine stones. This diffiuclty had actually been overcome one year earlier. K. A. H. Mörner (60) had discovered cystine in protein, and found hydrolyzed hair to be an especially rich source. The correct chemical formula of cystine (II), and its reduced form, cysteine (I) was established in 1902 by Friedmann (31):




I II

Chemical methods to measure urinary "neutral sulfur" (largely cystine) and inorganic sulfate were developed, and the first balance studies on the sulfur metabolism of a cystinuric were reported in 1905 by Alsberg and Folin (3).

But the chemical and the biochemical results produced mystification, not enlightenment. Loewy and Neuberg (54) reported that hair and stone cystine were metabolized differently and were different chemical substances. Alsberg and Folin promptly reported their own and others' reexaminations of the evidence for this conclusion (3): "After having read the above papers, one of us (F.) immediately examined a small stone recently passed by our patient." He could not verify such a difference (which hinged on a different crystal form). But as late as 1927, as a result of analysis of the 78 g. of stones from Tennant's case (79), this error found confirmation (37). The problem of determining the chemical identity of samples of cystine was difficult, but the full explanation of these conflicting results must consider the psychological difficulties produced in the minds of investigators by the curious results from metabolism studies on the cystinuric patients.

The metabolic studies firmly established that an increase in food protein produced an increase of the cystine excreted. But when the food protein was hydrolyzed outside the body and the isolated cystine was given to the patient, that cystine was oxidized to sulfate. Cystine itself, even cystine isolated from the patient's own urine, (80) caused no extra cystine to be excreted. The psychological effect of this paradoxical result, which was amply verified, was compounded by the results of Loewy and Neuberg. They confirmed the above results with stone cystine, which was oxidized to sulfate, but cystine isolated from hair was excreted as cystine! Garrod expressed the pessimism of the time (33): "The study of the metabolic peculiarities of cystinurics has yielded results which are very difficult of explanation, and the more the problem is investigated the more remote seem to be the chances of its satisfactory elucidation. Thus, in every case tested, except that of Loewy and Neuberg, in which cystin has been given by mouth, it has been completely destroyed, although the patients were all the time excreting cystin as such."

During the same period other observations were made from which our modern understanding of cystinuria could have been deduced, had another Niemann collated the findings. But like a hank of string with the key loop in sight, several pulls at other loops resulted in a snarl which took nearly fifty years to untangle.

## V. REWRITING HISTORY

During the first third of the twentieth century what had been sharp differences of opinion about the nature of cystinuria degenerated into an intellectual muddle. Further progress occurred only later, after time had dimmed the memories of facts and irreconcilable conclusions. Then a single group, led by Brand, redid the work. No attempt has been made till now to analyze the muddle left behind. It may be that such a state of affairs was inevitable, given the results of Loewy and Neuberg and the distraction of Abderhalden's new disease. Or the primary fault may not have been the handling of alleged facts, but the inadequacy of the concept of metabolism compartmented into exogenous and endogenous. If so, the scientific method may be expected to fail on occasion. It may help in thinking about this momentous question to review the positive experimental results available in Garrod's time. Through the retrospectroscope, clearly illumined by our present knowledge of cystinuria, certain pivotal findings from the turn of the century stand out.

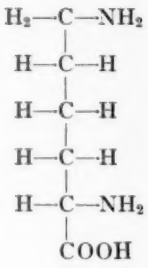
The enzymic apparatus for cystine degradation was obviously intact, since cys-

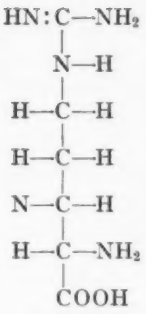
tine given as such to a cystinuric was excreted as sulfate. Any amount given orally could be oxidized by the liver, but if cystine or cysteine was given parenterally, to dogs or rabbits (7) or to a cystinuric patient (85), it was excreted by the kidney as cystine. Failure of tubular resorption, or in the language of that day, a low renal threshold for cystine, was later implicated quite directly by the fact that the level of cystine in the blood of cystinurics was not elevated (15). This eliminated the possibility of a renal overflow mechanism, although it was apparently not appreciated by the authors at the time. The meaning was perhaps not lost to all, however, for in 1934 a clinical case report cites a personal communication from "Dr. [I. M.] Rabinowitch of Montreal" that the mechanism of cystinuria is "analogous to the glycosuria in renal glycosuria . . . as a result of an undue permeability of the kidney . . ." (66). There was left unexplained then, and now (23), only the reason why the kidneys were presented with a greater load of cystine for excretion when protein was fed than when cystine was fed as such.

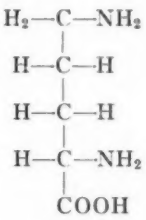
An even earlier series of experiments would seem to lead to the recognition of the other amino acids (lysine, arginine and ornithine) besides cystine that were present in abnormal amounts in cystinuric urine. This discovery in recent times immediately caused the realization that it was the kidney function and not cystine metabolism that was deranged. Ellinger (27) in 1898 found that bacteria would decarboxylate lysine and ornithine to form the foul smelling "ptomaines" or diamines, cadaverine and putrescine. These substances had been isolated from the urine of many (3, 74), but not all, cystinurics. The original patient studied in 1889 (82) had cystitis, and successive investigators over several years found the amounts of diamines increasing in pace with her urinary infection (36). These findings gave rise to the belief that intestinal infection leading to putrefaction was the basic abnormality in cystinuria (82, 83), supplanting the theory that it was a metabolic anomaly like diabetes and gout (74). When Moreigne in 1899 cast doubt on the possibility of a life-long intestinal infection and found that the cystine excretion was not altered by antiseptic treatment of the intestine, the theory was altered to call for a generalized disturbance of protein hydrolysis in the gut. Before even this theory could lead to the recognition of the other amino acids in the urine and their decomposition by bacteria to the diamines, Loewy and Neuberg supported it with evidence of quantitative excretion of the wrong amino acids, tyrosine and aspartic acid (54) (their third result in a single paper which has remained unique among the studies of cystinuria). It is interesting to compare the above results with those from the patient studied more recently by Harris, *et al.* (40) This cystinuric was exceptional among their patients in excreting no lysine. This led them to make a closer examination of the patient, which disclosed the presence of a urinary tract infection, and further analysis of the urine revealed the missing lysine present as cadaverine.

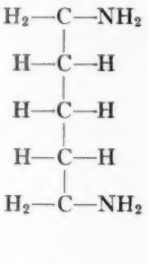
The particular findings of Loewy and Neuberg were tested immediately. Alsberg and Folin reported in the following year (1905) that hair cystine was not excreted as such, but as sulfate; cadaverine and putrescine were not excreted (there was no infection); aspartic acid was completely metabolized. The refutation was complete: "The more we have studied the experimental data presented in the paper of Neuberg and Loewi the less reason have we had to question the accuracy of the work except

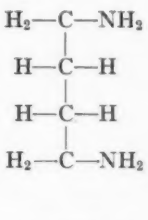
Cystine


Lysine


Arginine


Ornithine


Cadaverine


Putrescine

in one or two minor particulars. Our own experiments to be described in this paper have, however, entirely failed to corroborate their findings." (3).

It is now almost inconceivable that the work of Alsberg and Folin in 1905, quoted above, and that of Wolf and Shaffer in 1908, did not quickly solve the problem of cystinuria. The former were justifiably confident in their newly developed chemical methods, especially the chemical determination of cystine, based on the assumption that it comprised the neutral sulfur fraction. They also tested the theory of defective hydrolysis of dietary protein by measurement of the "undetermined nitrogen" fraction of urine, that part of the total nitrogen left after subtraction of that in urea, ammonia, creatinine, uric acid and cystine. In their patient there was an abnormal excretion of the undetermined nitrogenous substances and of cystine in starvation, and therefore these were produced by the endogenous metabolism. But both abnormal excretory products also increased in proportion to the protein in the diet, and therefore were produced by the exogenous metabolism. The concept of two separate metabolisms nevertheless survived for another generation, although Folin did not work again on cystinuria himself.

Four years later Wolf and Shaffer (85) confirmed the results of Alsberg and Folin in detail, and went on to build on the new discovery made by Alsberg and Folin: that there was an abnormal excretion of the "undetermined nitrogen" fraction. The results were clear-cut and the conclusions sound, even though they were not

effectively developed until forty years later: "The high undetermined nitrogen is in part due to cystine, and is in part due to other amino acids; for the ratio of amino-acid nitrogen to neutral sulfur is much above that found in normal subjects. Cystinuria is probably never a simple anomaly in which the cystine complex is the only part of the protein molecule which is affected. Owing to the difficulty of their separation, it is impossible to say what are the other fractions which are concerned in the increase in the undetermined nitrogen (85)." Somehow, this clear statement was forgotten. Perhaps it was due to lack of good methods to extend it. Later isolations of crystalline lysine derivatives from cystinuric urine by Ackermann and Kutscher in 1912 (2) and by Hoppe-Seyler in 1933 (43) passed almost unnoticed and perished for lack of corroboration. The latter obtained amounts equivalent to 0.1 g. lysine per liter of original urine, which without allowing for losses, was still grossly more than could be present in normal urine. The discovery of three other amino acids besides cystine in cystinuric urine forty years later surprised that part of the medical world who heard of it.

This abstraction of pertinent findings and clues out of the context of the times makes it natural to wonder, given the observations repeatedly made, why early investigators did not deduce the real nature of the dysfunction in cystinuria. But the eradication of all the wrong results and of inadequate theories would not necessarily have advanced the field in the long run. Such action, for example, would have eliminated Garrod's concept of an inactive enzyme: in many ways an inadequate theory based on wrong facts, but one which was responsible for greatly furthering the understanding of cystinuria and hereditary disease in general.

## VI. GARROD'S "INBORN ERRORS OF METABOLISM"

The brilliant concept of the inborn errors of metabolism was enunciated by Garrod at the height of the confusion about cystine metabolism (34). According to this thesis, an hereditary condition characterized by the accumulation of an abnormal metabolite resulted from the hereditary lack of an enzyme that normally removed that metabolite. Here was an illuminating way to regard the accumulation of cystine, which in a normal individual was oxidized to sulfate. Cystinuria was included as one of the inborn errors of metabolism, and lent its weight to this important concept. For a generation the fact was obscured that the supposedly missing enzyme reaction in cystinuric patients was in fact not missing. The reaction had been demonstrated equally well in both normal and cystinuric subjects. In either kind of subject cystine administered as such orally was oxidized and excreted as sulfate. The defect in cystinuria was manifested only if some precursor of cystine, such as protein, was fed. Garrod was not unaware of this paradox, and his original lectures and later revision of them are valuable summaries of the research on cystinuria (32, 34), but he contented himself with the explanation that the metabolic error was partial and incomplete, and that the contradiction would be resolved when more was known of the intermediary steps of sulfur metabolism.

The major contribution of Garrod's thesis specifically to the study of cystinuria was to supply a mechanism accounting for the familial distribution of a highly uniform and subtle disorder. In addition to the occurrences of cystinuria in siblings

mentioned by Niemann, and omitting the cases of familial cystinosis described by Abderhalden, there was ample evidence of its hereditary nature. Pfeiffer (67) had reported four cystinuric children of parents who were first cousins. Cohn described a family with a cystinuric mother, a normal father and ten children. Six of the children were cystinurics, two definitely not, and two untested (17). Two of the children were twins, both cystinurics. Identical twins, both with the disease, who also developed stones almost simultaneously were later described by Kretschmer (47) and in another instance by Harris and Warren (41). Garrod saw that this pattern was meaningful: homozygotes for a rare recessive gene would usually occur among siblings, rarely in other generations of the same family, and more commonly in families where a consanguinous marriage improved the chances that both parents would possess the rare gene. He did note, however, that there was a greater frequency of direct transmission of cystinuria from parent to child than was met in connection with the other metabolic errors he described (i.e. albinism, alkaptonuria and pentosuria). Fifty years later (40) the recognition of a "dominant" form, manifesting cystinuria in the heterozygote, confirmed his impression.

It was fortunate that certain substances giving rise to cystine in the body, such as dietary protein, caused excretion of extra cystine, just as other precursors caused increased formation of the blocked metabolite in the other errors of metabolism where degradation was blocked by enzyme deficiencies. For this reason the concept of Garrod had pragmatic consequences for biochemistry in connection with the intermediary steps of sulfur metabolism, in addition to its genetic implications. The next advance in the study of cystinuria exploited this excretion of cystine formed from precursors *as if it were the result of an enzyme deficiency*. In the same way that the defect in alkaptonuria was used to determine the pathway of the tyrosine precursors of homogentisate, the non-existent enzyme defect in cystinuria was used to reveal the complex pathway of sulfur-containing amino acids.

## VII. DOWN THE METABOLIC PATH

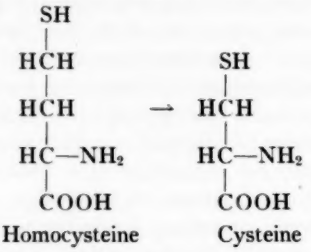
In 1935 the biochemist Brand and the urologist Cahill, with two young cystinuric patients, began to elucidate the metabolic pathway of the sulfur-containing amino acids by feeding experiments. The erroneous assumption of a block in cystine metabolism was pragmatically useful. The identification of cystine precursors was equally well served by the renal leakage of the extra cystine as it would have been by the supposed metabolic block. Cysteine and the new sulfur-containing amino acid, methionine, were promptly identified as cystine precursors (12). Proteins containing more methionine gave rise to higher cystine excretion than proteins with low methionine content. The cystine contained in the protein was oxidized to sulfate. This identification of methionine, and not cystine, as the principal dietary source of urinary cystine was confirmed by Lewis (52).

During the two years of experimentation the subjects of Brand's experiments (who were thanked in each paper) formed extra cystine from homocysteine, but not homocystine (10) (cf. cysteine but not cystine), and not from a number of other possible sulfur-containing compounds. Brand postulated that cysteine was a product of the catabolism of methionine and that the error in cystinuria was a failure of the

proper utilization of cysteine, not a failure of cystine metabolism. The final results clearly indicated a pathway of metabolism of sulfur-containing amino acids with the surprising sequence of reactions:—(12)

Methionine → Homocysteine → Cysteine → Cystine → Sulfate
↓ Urinary Cystine (in Cystinuria)

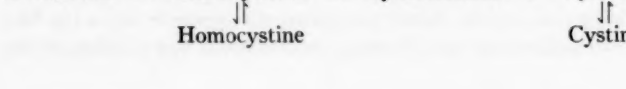
The reaction difficult to believe was the shortening of the carbon chain in the conversion of the $\gamma$-thio compound, homocysteine, to the $\beta$-thio compound, cysteine:




Lewis confirmed the essential findings for this scheme, that methionine and cysteine gave rise to extra cystine, but he refused to accept the same conclusion. His attitude was more cautious, but it was still reminiscent of Baumann's scepticism that an alkaptonuric patient would be able to convert a 4-hydroxyphenyl- to a 2,5-dihydroxyphenyl compound (because this was a difficult chemical change, Baumann said that homogentisate must be formed from tyrosine by intestinal bacteria (46)). Lewis rejected Brand's scheme involving the shortening of a carbon chain because: "It is difficult to picture such a transformation of homocysteine to cysteine and to cystine in the light of any known facts concerning the degradation of the biologically important sulfur-containing compounds either *in vitro* or *in vivo*. Until further evidence is available we are unwilling to accept such a theory of the origin of the urinary cystine, although we are not prepared to suggest an alternative theory by which the increase in cystine excretion, which results from methionine feeding, may be explained." (52).

Brand's scheme (9) was confirmed in detail, however, and with it the validity of the use of a patient with cystinuria for these purposes, by the studies from du Vigneaud's laboratory. $S^{35}$ labelled methionine gave rise to labelled cystine, but $C^{13}$ labelled methionine did not (24). The shortening of the carbon chain referred to above occurred by transfer of the sulfur from the 4-carbon compound, homocysteine, to a different 3-carbon compound, serine, to form cysteine made up of the original sulfur but with new carbon atoms. The intermediate of this interchange was cystathionine, a combination of both homocysteine and serine in one molecule. This compound, too, was later shown to give rise to cystine (68).

After the pathway of sulfur amino acid metabolism in man was delineated by



Brand through his studies of a patient with the hereditary condition of cystinuria, a very similar pathway (in the reverse direction leading to methionine) was proved for *Neurospora* by the use of several mutants of this species (44). The sequence of these discoveries provides another instance to illustrate that what is now called biochemical genetics was effectively begun by observations on human material by the physician Garrod.

*Cystinuria in Animals:* Of the "inborn errors of metabolism" enumerated by Garrod, only two, albinism and cystinuria, have definitely been recognized in animals. This promising field of research is still largely unexploited, but what has been done was started in connection with Brand's work just described.

The sporadic occurrence of urinary cystine stones in dogs had been reported earlier, first from Paris in 1823 by Lassaigne (48), again in 1861 (38), and in a male Dachshund in 1921 (49). But always the stone was recognized after the death of the animal and no metabolic studies had been made. The tendency to form cystine stones was not associated with a single breed, as was the frequent development of urate stones in the Dalmatian coach hound. Then in 1935, Morris, a veterinarian, found an Irish terrier suffering from urinary retention to have cystine crystalluria. At operation several vesicular and urethral calculi of cystine were successfully removed (61). Brand and Cahill joined the study, and undertook the necessary chemical studies to identify the disease and arranged a breeding program to investigate the genetics of the condition.

The project apparently languished and the dogs were dispersed sometime before Brand's death in 1953—just before the new concept of the renal tubular mechanism in human cystinuria could be tested on cystinuric dogs. Only fragmentary reports of this important study were ever published. Some old-style metabolic studies of the dogs were recorded, and these nearly duplicated the findings from contemporaneous human studies. Cystine accounted for 13 to 17 per cent of the total urinary sulfur in the cystinuric dogs (13, 35), compared with about 20 to 30 per cent in the human cystinuric (52). Brand's metabolic scheme for sulfur-containing amino acids was supported by the excess cystine excreted when the dogs were fed extra protein, methionine, or cysteine. The feeding of cystine did not result in extra cystine excretion (42).

Through the American Kennel Club registrations of the sire and dam of the first cystinuric dog, four related dogs were found to begin a breeding program. One, a cousin of the proband, also had cystinuria (11). Twenty-five unrelated animals of the same breed were not cystinuric. Three hundred dogs of the cystinuric strain were raised, and twelve cystinuric animals were identified. All affected animals were males (8, 13). The original cystinuric male produced litters with an unrelated female and with two half-sisters. None of the 15 pups was cystinuric (35). No genetic analysis of the results was ever made, but the few published pedigrees resembled, or at least did not exclude, a sex-linked mode of inheritance (13, 42), one quite different from the recessive mode seen in man.

It remains to be shown, when cystinuric dogs are found again, that a similar discrete renal transport system under hereditary control is affected in the cystinuria of both men and dogs. It is important to establish whether or not other amino acids

besides cystine are excreted in abnormal amounts by the cystinuric dogs. It was in normal dogs that the competition between the several different amino acids for renal tubular excretion was first demonstrated (6), and on this was initially based the belief that the excretion of the several amino acids in man was due to a deficiency of a single renal transport system. But the apparently different mode of inheritance in dogs, coupled with the existence in another species (see below) of cystinuria without other aminoaciduria, suggests that canine cystinuria may only be a phenocopy of human cystinuria.

The second species of animal with cystinuria is an obscure creature, the blotched genet, a kind of wild cat from Kenya. In a survey by paper chromatography of the amino acids excreted by different animals at a zoo, Datta and Harris (18) found every blotched genet examined to be cystinuric This condition was present in a number of unrelated animals, so it was not just a familial condition *within* the species, but a species characteristic. It was perhaps fortuitous that Harris, like Brand, was also working on cystinuria in man before making this discovery.

The cystinuria of the Kenya genet differs from that in man in that there is no excretion of high concentrations of other amino acids. Additional studies cited in brief (22) established that the cystinuria occurred by a renal mechanism, i.e. a deficiency of tubular reabsorption. Strangely enough the concentration of cystine in the genet urine was said to be about 1 to 2 mg/ml *in true solution*, that is, about four times its solubility in human urine. Yet the genets do not form urinary cystine stones. The failure of cystine to precipitate from these urines raises again the possibility of a cystine-containing complex existing in human (14, 5, 12) and dog (42) cystinuric urines, although the existence of such a complex has been emphatically denied (52). The early belief in such a cystine complex may have arisen from a theory that when cystine was absorbed as a peptide, because of faulty digestion, it escaped oxidation in the tissues and produced cystinuria (72). No stable complex of cystine was noted in genet urine, at any rate. The unusually high urea concentration of 10 to 20 g. per cent in genet urine, and the absence of other amino acids (16) might affect the solubility of cystine. Many questions of physical chemistry and physiology, and perhaps even therapeutic possibilities for cystinuria in man, are raised by these high urinary levels of cystine, but all must await further studies of the condition in the Kenyan genet.

Very recently cystine urinary stones have been discovered in another animal species, the mink (65). The economic importance of this animal should guarantee that the academically important questions about an analogue of a human disease will be answered. Already other mink with the disease have been found among those related to the initial case (personal communication).

### VIII. THE BREAK-THROUGH: PATTERN OF AMINOACIDURIA

For a decade after Brand's work on cystinuria no further advances were made in the understanding of why cystine was excreted by the patients with this disease. Then, from a most unexpected quarter, came decisive and revolutionary findings which altered the very definition of the disease. In 1947 microbiological determinations of the individual amino acids in the urine of a cystinuric girl (compared with

the results of seven female controls) showed a high cystine excretion, but also arginine and lysine excretions about ten times the normal level (87)! These abnormalities in the excretion of other amino acids at last freed investigators from the bias imposed by the clinical nature of the disease. The unique insolubility of cystine caused its crystallization in nearly pure form in the urinary tract, as if it were the only substance present in abnormal amounts. The earlier methods which had given suggestions that other substances were present had not been sufficiently reliable to destroy this bias. But the specificity and quantitative accuracy of the microbiological methods now used at once established the new findings as fundamental characteristics of the disease. Equally powerful and even more convenient methods of amino acid analysis soon confirmed these new abnormalities.

The first explanation of the large amounts of two new amino acids found in the urine of cystinuria was progressive renal damage, producing first the leakage of these few amino acids and later, perhaps, the generalized aminoaciduria seen in Fanconi's syndrome (i.e. Abderhalden's disease, *v. supra*) (87). However, long clinical experience belied this. A non-specific renal damage would not show such specific leakages. Column chromatography of the urinary amino acids in six cystinuric patients, all with histories of cystine stone formation, showed that a definite pattern of aminoaciduria was characteristic of this disease (77). In addition to the cystine, lysine and arginine already identified, ornithine, a relative and associate of arginine in the urea cycle, was also found in abnormal amounts. The remaining amino acids were not different from normal. Moreover, in each of five patients, the abnormal amino acids were excreted in similar relative amounts, approximating the molar ratio of 1:1.3:2:5 for ornithine-cystine-arginine-lysine. While the constancy of these ratios was to some extent fortuitous and determined by the particular patients chosen (40), it emphasized the specific nature of the lesion in cystinuria. Stein suggested that instead of a renal tubule defect (77), a metabolic defect, enzymic in nature but probably in the kidney, simultaneously affected the reactions of all these different amino acids.

*Renal Physiology:* The possibility that a discrete renal tubular transport mechanism with enzyme-like specificity could be defective in cystinuria and give rise to the specific aminoaciduria was first appreciated in 1951 by Dent and Rose (21). It was known that arginine and lysine competed for the same tubular reabsorptive mechanism in the kidney of the dog, mutually lowering their Tm's (6), and it was suggested that this mechanism was missing in cystinuria. The first evidence to substantiate this was an elaboration of an old finding, by Brown and Lewis in 1937 (15), that the level of cystine in blood of a cystinuric was not elevated. In ten normal and nine cystinuric individuals the level of cystine in the plasma averaged 0.82 and 0.69 mg. per cent, respectively (29). It was argued that if cystinuria was the result of a renal defect, the plasma level would be normal or low as it appeared to be, while if it was the result of a blocked cystine metabolism, the plasma level should have been elevated for excretion to occur. This logic omitted from consideration the most recent hypothesis (Brand), that defective utilization of cysteine, not cystine, was the cause of cystinuria. That theory died unattended during the rush of the new advance. Evidence was presented that the levels in blood were normal

for the other amino acids also excreted in cystinuria (20). It was unlikely anyway that the several amino acids would share a common metabolic reaction, and the data on blood levels made it increasingly unlikely that any single metabolic block could be responsible for the disease.

Still, the crucial evidence for a specific renal mechanism required that the renal clearance of the amino acids be measured, and that the normally low clearances be found elevated, nearly to the glomerular filtration rate, by the loss of the ability of the tubules to reabsorb the affected amino acids. Exactly these results were found for cystine in a classical study by Dent, Senior and Walshe (23). Cystine clearance in normal individuals was of the order of 4 ml/min. In two cystinuric patients (both with considerable renal damage from stone formation and therefore probably with reduced glomerular filtration rates) the cystine clearances were about 100 ml/min., i.e. they approached the normal glomerular filtration rate. Moreover, in a person known to be heterozygous for the cystinuria gene, a definitely elevated cystine clearance (14 ml/min.) was found. It remained to be shown, only for completeness, that similarly elevated clearances existed for the other amino acids excreted in abnormal amounts.

Elevated renal clearances in cystinurics of cystine, arginine, ornithine and lysine were then demonstrated by Robson and Rose (71). The clearances of cystine, arginine and ornithine were not further increased by the rapid intravenous infusion of 5 g. of L-lysine, because the clearances were already near the glomerular filtration rate and there was little or no tubular reabsorption to be inhibited. In normal individuals and heterozygotes of cystinuria there was an active reabsorption system to be inhibited by the load of lysine. The lysine, because it normally shared a common stage in reabsorption with the three other amino acids, caused in the heterozygotes transient increases in the clearances of the three other amino acids, and only of the three.

The general nature of renal tubular transport systems of the kind at fault in cystinuria had been defined in experiments similar to those given above by earlier workers in the field of renal physiology. Shannon had shown that xylose, for example, with one less carbon than glucose but with the same configuration as in the first five carbons of glucose, was reabsorbed by the same tubular mechanism as was glucose. This transport system had not only enzyme-like substrate specificity, but related substrates would competitively inhibit it. Other substances were known to share still different transport mechanisms. The total action of an undetermined number of such discrete mechanisms accounted for tubular function. At some one step in tubular reabsorption, a specific reversible combination between an element of the transport system and the substance being transferred must occur (73).

Cystinuria could then be defined as an hereditary inactivity of a specific renal transport system which normally combined with and reabsorbed the several related dibasic amino acids. The inactivity would result from the absence or defect of a specific enzyme-like combining substance in the tubular cells. More or less complete inactivity of the system caused clinical signs, and occurred in individuals with two genes for the condition. But even the heterozygote, the carrier of a single gene who had no clinical signs, showed the stigma of this gene, in the one individual

tested, by a moderately reduced cystine reabsorptive ability because his tubule cells were partially deficient in the specific transporting substance under hereditary control.

## IX. AN HEREDITARY BIOCHEMICAL DEFECT

While the group led by Dent at University College, London, was developing the renal explanation to replace Garrod's theory of an hereditary metabolic block in cystinuria, another group under Harris at the same institution used the methods of genetics to define the lesion of cystinuria in considerable detail. From these combined studies emerged at last the picture of an hereditary biochemical defect with the specificity envisaged by Garrod, but affecting an enzyme-like transport system instead of a metabolic enzyme. Thus the solution of this problem forced an expansion of Garrod's original concept to include hereditary defects in other functional systems besides enzymes.

When the diagnosis of cystinuria had depended almost solely on the appearance of stones, the familial incidence of the disease among siblings was such that Garrod correctly suggested its inheritance through a rare recessive gene. Even then, however, too many instances of direct transmission from parent to child were known, and Garrod entertained the possibility that the condition might be dominant. An alternative explanation, that the gene was very common instead of rare, would also account for the presence of the disease in two successive generations. An individual with the disease would then not uncommonly mate with a heterozygote (carrier) and the disease would reappear among the offspring of the mating. This explanation was possible only if cystinuria was much more common than had appeared from the incidence of stone formation. Indeed, chemical tests for urinary cystine revealed a much greater incidence of cystinuria (1:600) than had been suspected on the basis of stone formation or crystalluria (1:10,000 or 20,000).

*Incidence of Cystinuria:* Between 1929 and 1931 H. B. Lewis examined the urine of 10,534 Michigan University students with the cyanide-nitroprusside test, and when that was positive, by the more specific Sullivan test for cystine (51). The normal excretion of cystine is in the range of 40 to 80 mg. per day (69) and about the same amount per gram of urinary creatinine (41). It has been estimated that Lewis' cyanide-nitroprusside test would be distinctly positive with urines containing 200 mg. cystine per day (19) and positive with at least 100–125 mg. cystine per gram of creatinine (41). In the large population tested Lewis discovered eighteen individuals, or 1 in 600, who consistently excreted cystine. Eleven of these were studied in more detail and all showed an abnormally elevated urinary organic sulfur fraction. Four of them showed repeated cystine crystalluria (4 in 10,000). Earlier estimates of the incidence of cystine crystalluria were less than one-fourth this high, but these estimates were based on single examinations for a condition known to occur only intermittently. None of the individuals found by Lewis had ever had symptoms suggestive of renal stone or colic and only one gave a family history of such disease. A sister of one of the individuals had formed a cystine stone, but his parents and five other siblings did not excrete cystine. The urine of twenty-two other individuals in the population studied gave weakly positive tests consistently

or occasionally, but all had nearly normal sulfur partitions, and were considered normal. Malleson (cited in 41) confirmed the high incidence of chemical cystinuria, with the finding of four positive urines from about 1,000 students at University College, London. These urines contained 106 to 161 mg. cystine per gram of creatinine, and paper chromatography demonstrated in all of them an increased output of lysine as well as cystine.

It was immediately apparent that only a small fraction of the cystinurics that could be diagnosed chemically would form stones. But prior to this time, the best evidence was consistent with Niemann's belief that almost all cystinurics (diagnosed by crystalluria) ultimately formed stones. Since stone formation occurred randomly at all ages, it was reasonable to suppose that about half of all the cystinurics in the population at a given time had already formed stones and the other half eventually would in the future, an incidence of stones in cystinuria of about 50 per cent at any given time, although this estimate was never explicitly stated. On the other hand, a curiously different estimate, that only "2½ per cent of cystinurics develop stones" (26, 28), crept into the medical literature about 1923 and was widely repeated without question (62, 84) (see (70) for other references to this myth). It was probably derived from a misinterpretation of some statistics giving the percentage of cystine stones found among all urinary calculi (see (58)). This figure, though pulled out of the air, does happen to be the approximate frequency of stones among chemically detectable cystine excretors, but was cited before Lewis had discovered how relatively common chemical cystinuria was. Lewis commented wryly on this recurrent error that anticipated his discovery, that since cystinuria "was diagnosed almost invariably in connection with the presence of calculi, the basis of such an estimate is not clear to the present author" (51). Lewis' new evidence, that chemical cystinuria was relatively common, while cystine stones were rare, demanded that an additional criterion, apart from stone formation, be developed to identify the specific disease in stoneless people and distinguish it from other conditions where cystine excretion might occur. Twenty years later the specific aminoaciduria of four amino acids provided the necessary criterion for recognizing cystinuria in the absence of stone formation.

The indicated investigation was promptly undertaken by Dent and Harris (19). No further purpose will be served by using the term cystinuria in a general sense, as they did, to describe abnormal amounts of cystine in the urine from whatever cause. The result of their study was simply to redefine cystinuria as the specific condition characterized by the presence of the four amino acids in abnormal amounts, and to prove it was distinct from the Fanconi syndrome and from Wilson's disease. In the latter diseases cystine excretion might occur as part of a general aminoaciduria. The families of three individuals with the latter two diseases contained three other individuals with a similar general aminoaciduria and none with the specific cystine-arginine-lysine type of aminoaciduria. The families of seven classical cystinurics, six of whom had stones, contained seven more individuals of the same specific type and none with the generalized aminoaciduria. These findings directly disproved the idea that cystinuria was a stage in the development of the Fanconi syndrome

(87), which had subsequently been taken up by other authors without further proof. The findings proved each of these diseases to be genetically independent.

*The Pattern of Inheritance: Questions and Answers:* The incidence of cystinuria found in this study, which included seven new cases from seven propositi, was not different from that expected on the basis given above, that about equal numbers with and without stones would be found at a given time. But even twice the number of total cases as were known by stone formation could greatly alter the apparent pattern of inheritance. From a consideration of the published pedigrees of cystinuria, complete except for those given by Niemann (64), Kretschmer (47) and Mörner (59), Dent and Harris concluded that the available data was of little genetic value since only five families had been examined with chemical methods. The diagnoses could all be questioned because none of these family studies gave information about the excretion of other amino acids. The chance of this diagnostic error would seem to be very small, given the presence of cystine urinary calculi in a family. But the inclusion of Abderhalden's cases of familial cystine diathesis among the cystinuric pedigrees reviewed by Dent and Harris themselves stood in mute testimony of this danger. The data from the earlier studies simply re-emphasized the mutually contradictory points made by Garrod: the frequency of parental consanguinity and of cases among siblings indicated that cystinuria was a homozygous recessive disease; but the presence of affected individuals in three successive generations of the same family (the example of Andrews and Brooks, a cystinuric family tree of twenty-five, seven of whom were cystinurics (4), can replace the dubious one of Abderhalden) almost certainly indicated that some patients in this family were heterozygotes, i.e. the condition was sometimes dominant.

Analysis of the new family data collected by Dent and Harris did not resolve this problem. In two instances the parents of cystinurics were normal, so the condition was not regularly manifested in heterozygotes. The occurrence of affected parent-child pairs in two of the seven families could be explained on the recessive hypothesis in view of the postulated high frequency of the gene in the population (one individual in twelve would be a heterozygote if Lewis' incidence of 1 in 600 represented the homozygous form). But if the gene were so common, consanguinity would not be important for its expression. Yet even in this small series there was one consanguinous marriage. It was apparent that the genetics of cystinuria, half-known for so long on the basis of the qualitative excretion of cystine, would yield fully only to a detailed quantitative study.

Quantitative studies by Harris and coworkers of the amino acid excretion in cystinuric families provided the answer in two years' time. Initially, the quantitative measurements of cystine excretion in twenty-one families (41) showed only in some of the families the expected clear separation into cystinuric and normal individuals. In the other families, there was a continuous gradation from the normal to the extremely abnormal amounts of cystine excretion in the different individuals. It was evident that the disease appeared in two forms, separately inherited. Type I, the more common of the two, appeared in families where all individuals excreted either normal or highly abnormal amounts of urinary cystine, and no individuals

had intermediary values. There was a sharp segregation for the property of cystine excretion, and the family distribution was of the type expected if the cystinuria occurred in individuals homozygous for a rare recessive gene. The parents, who were apparently normal, were necessarily heterozygotes. But the character of the one recessive gene which they possessed was not strong enough to create any noticeable effect on cystine excretion. Thus, the defect appeared only among the homozygous offspring. Four instances of consanguinity in these families further supported this view.

In families of Type II, normal, intermediate and high values of cystine excretion were all found. The family distributions suggested that the individuals with moderately raised values of cystine excretion were heterozygous and those with high values, like the individuals in Type I families, were homozygous for a single rare gene. Because the character of the "incompletely recessive" gene in Type II families was strong enough to produce intermediary values in the heterozygotes, it was assumed that the individuals with normal cystine values did not possess the gene for cystinuria. Whether two distinct diseases or two variants of the same disease had been discovered could only be answered by further quantitative studies of the other amino acids excreted, but it was clear that the earlier contradictory results had found their explanation in two different modes of inheritance of what appeared to be the same renal defect.

*Two Variant Forms of Cystinuria:* The variant forms of the disease originated in inheritance of different degrees of the renal defect in cystinuria, as was clear from study of the lysine and arginine excretions (39). The number studied included twenty-eight stone-forming cystinurics and 121 of their relatives, undoubtedly the largest collection of cystinuric families ever observed by a single group of investigators. As before all degrees of cystinuria from 20 to 800 mg. of cystine per gram creatinine were found. Only in individuals excreting 250 mg. cystine per gram creatinine or more had stone formation occurred. This was the level at which a saturating concentration of cystine in urine, about 300 mg per L, could be reached and precipitation would be likely to occur. The lysine excretion was regularly about twice that of cystine and paralleled the cystine excretion throughout all its gradations. Arginine and ornithine, however, were preferentially reabsorbed by the tubular mechanism in question. These amino acids were not excreted in abnormal amounts until the cystine and lysine reabsorptions were seriously impaired. Thus a genetic study revealed the hierarchy of affinities of substances for a renal physiologic mechanism. At a cystine excretion of 250 mg. per gram creatinine, where stone formation became likely, arginine and ornithine also appeared in the urine in abnormal amounts and increased proportionally with the excretion of higher amounts of cystine and lysine. The genetically determined degrees of the renal defect, if partial, permitted the preferential reabsorption of arginine and ornithine, and if more complete, resulted in the renal loss of all four amino acids, including cystine in sufficient concentration for it to precipitate and form stones.

The biochemical and genetic data of the preceding studies (39, 41) were finally correlated in a definitive paper on "Phenotypes and Genotypes in Cystinuria" (40). A total of twenty-nine families, each with at least one stone-forming cystinuric

member, had been studied. Two of these families could not be definitely categorized as Types I or II from the data available, but in all families the homozygous individuals were indistinguishable. They excreted approximately the same highly abnormal amounts of the four amino acids, and nearly all formed cystine stones. In nineteen of the families (Type I) the condition was recessive and no degree of abnormality was found except the extreme form in the homozygotes. In eight families (Type II) the condition was "incompletely recessive," and the heterozygotes showed moderately increased excretions of cystine and lysine without increased excretion of arginine or ornithine. Only in rare instances did the latter excrete sufficient cystine to form stones.

Implicit in these figures of the relative frequency of the two types, with about one-third of the families manifesting cystinuria in the heterozygotes, was the explanation of the rarity of cystine stone and the commonness of chemical cystinuria. Accepting a conservative order of magnitude for Garrod's estimate of the incidence of cystinuria with stones or crystalluria as 1 in 20,000 people in the population, it is indeed a rare condition. Only one-third of these, or 1 in 60,000 people, would then represent the still rarer type whose heterozygous relatives would show chemical cystinuria without stones. Yet the astonishing frequency of the heterozygotes of a rare homozygous condition is such that, even with this degree of rarity, it would be easy to account for the observed incidence of chemical cystinuria. The frequency of heterozygotes of the rarer form would be about $2\sqrt{\text{incidence}}$ of the homozygous form, or 1 in 125! The incidences of chemical cystinuria observed by Lewis (1 in 600 consistently and 1 in 260 including the occasional excretors) or by Malleson (1 in 250) were less, and this suggests that cystinuria with stones is less than half as common as Garrod estimated. The "complete ascertainment" of Mörner of the incidence in Sweden would set the minimal incidence at 1 in 200,000.

*Alleles?:* An unsolved problem concerned the genetic relation between the two types of cystinuria, distinguishable only by whether or not cystine and lysine excretion occurred in the heterozygotes. Since the same renal system was affected in both types, and they differed only in the heterozygotes in regard to the quantitative effect on the renal system of the single gene, it is not likely that separate genes at different loci are responsible for the two types. Other considerations were also against this view (40, 41). A single mutant gene, accompanied in certain families by modifiers at other loci, possibly could give rise to the two observed types. The simplest explanation, however, would attribute the phenomenon to multiple alleles, that is, to at least two mutant conditions of one gene. One would have a mild and the other a severe effect on the tubular reabsorption of the four amino acids in the heterozygous state. Genetic proof of this reasonable possibility, Harris suggested, must await the identification of a homozygous cystinuric born of parents, one the apparently normal Type I heterozygote and the other a Type II heterozygote. Families ostensibly of this kind are on record, with one parent who excreted cystine but did not form stones, the other parent "normal", and a stone-forming offspring. But detailed chemical identification of the type of each individual in such a pedigree has not been done.

Direct chemical identification of the phenotypes of the parents of a stone-forming

cystinuric would now appear to be a possible and a more economical way of establishing the allelism of the two variant genes. Since one gene has a mild, and the other a more severe effect on the tubular reabsorption of cystine and lysine, the measured renal clearance of these amino acids should differentiate the two kinds of heterozygotes from each other, and distinguish both from the normal. At least, such measurements would identify the heterozygotes who should be subjected to more intensive genetic studies to decide this question.

*The Moral:* It appears that research on cystinuria had little influence on the conceptual thinking of the time, but was captive to the conceptual schemes of the times. The eventual demise of the concepts of "diatheses" of disease, of separate endogenous and exogenous metabolisms, and even of inborn errors of metabolism as exclusively enzyme deficiencies, appears to be unrelated to their unsatisfactory accounting for the facts of cystinuria. The popularity of the theories slowed the learning about cystinuria. If this relationship were true for other diseases, the medicine of the time would stand revealed as not a science, but a para-science, only borrowing and applying what it could, instead of producing its own conceptual schemes to account for its own phenomena. The scheme which today accounts for that rare curiosity, cystinuria, and other biological phenomena as well, could have been engendered by the findings in cystinuria, but was not.

A reluctance to give the facts about a rare disease primacy over the popular theories of the day may account for the remarkable periodicity of advance and stagnation in the history of ideas about cystinuria. The impetus from the initial discoveries in 1810 of the chemical cystine, and the disease, cystinuria, reached a surprisingly sophisticated level of understanding by 1823, when Prout wrote "la cause tient plutôt à l'action dépravée des reins qu'à une lésion générale du système." (70). The clinical definition of the disease, and the first pregnant statement about a block in metabolism had to wait for Niemann in 1876. Then shortly before 1900 began a burst of activity which produced the chemical structure of cystine, the best metabolic balance studies in cystinuria, and the concept of inborn errors of metabolism. A stalemate ensued. The power of Garrod's idea, that specific blockages of metabolism were inherited, prohibited the obvious conclusions from Folin's studies that the cystine metabolism was normal in the hereditary disease of cystinuria. Brand's work in 1936 represented the ultimate attempt to provide experimental supports for Garrod's concept. Some years after this agonal twitch in a dead approach, between the initial discovery by Yeh, *et al.*, in 1947, and the definitive paper of Harris, *et al.*, in 1955, the problem was solved. Cystinuria was an inherited defect of kidney function.

Garrod's concept of inborn errors of metabolism was not vitiated, as has been thought, because a specific renal tubular reabsorption system and not an enzyme under hereditary control was inactive in cystinuria. Any vitiation should more properly be attributed to the earlier recognition that the Dalmatian coach hound was unusual among dogs by excreting uric acid, not because he lacks uricase, which he does not lack (45), but because his renal tubule fails to reabsorb uric acid (30). On the strength of the modern findings in these two conditions in man and dog, conditions previously thought to illustrate the hereditary control of enzymes of

intermediary metabolism, it is clear that Garrod's concept should not be discarded, but must be extended to include the hereditary control of specific functional substances in the body other than enzymes. In the present instance the inheritable substance is an "enzyme-like" transport system in the renal tubule. It could be called a specific "here-to-there 'ase," to emphasize its analogy to the substances thought to be affected in the original statement of the inborn errors of metabolism. Into this enlarged scheme, engendered by the phenomenon of cystinuria, can be fitted immediately a number of other hereditary conditions characterized by "low renal thresholds," such as renal glycosuria.

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# The Distribution of The Sickle Cell Gene in Liberia

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WHEN ALLISON (1954) first advanced the hypothesis that the high frequencies of the sickle cell gene were to be explained by a balanced polymorphism in which the heterozygotes for the sickle cell gene possessed a relative immunity to falciparum malaria, he attempted to show that the tribal frequencies of the sickle cell trait in East Africa were all in agreement with this hypothesis. His own investigations among 35 tribes showed a remarkable correlation between the frequency of the sickle cell trait and the intensity of malaria. All tribes with relatively high frequencies of the sickle cell trait inhabited areas in which malaria was hyperendemic, while the tribes with relatively low frequencies inhabited areas in which malaria was either absent or not transmitted for several months of the year. Although at that time data on the frequency of the sickle cell trait in West Africa were very fragmentary, Allison (1954) indicated that, insofar as known, they were in agreement with his hypothesis. More recently Allison (1957) has shown that the presence of the gene responsible for hemoglobin C, which is an allele of the sickle cell gene, complicates the distribution of the sickle cell gene in West Africa. In populations with high frequencies of the hemoglobin C gene, there tend to be low frequencies of the sickle cell gene.

It will be the purpose of this communication to present data on the frequency of the sickle cell trait in Liberia, a region of West Africa previously unstudied in this respect. The finding of a relatively low frequency of the sickle cell trait here, in an intensely malarious region, indicates that factors other than malaria pressure must be invoked to explain the present-day distribution of the sickle cell gene in Liberia —the nature of these factors will be explored briefly.

## MATERIALS AND METHODS

The following data on the distribution of the sickle cell trait in Liberia were collected during ten months of field work, extending from September 1955 to August 1956. Most of the investigation was carried out at the Liberian Institute of Tropical Medicine, which is located near the seacoast about 40 miles southeast of Monrovia, the capital of Liberia. It is adjacent to the Firestone Rubber Plantation, which employs about 20,000 natives, among whom most of the tribes of Liberia are represented. The majority of the subjects tested were these workers or their families, but trips were also made to other parts of Liberia in order to test tribes which were poorly represented at the Firestone Plantation.

On most of these surveys sickle cell preparations were made and examined by the author in the field, but also included are the results of a survey on which blood was collected in 10 cc. "venules" containing oxalate and air-shipped to the Child Research

Received August 17, 1957.

Center, Detroit, Michigan, where a variety of studies were performed on the blood. Some of the results of the latter survey are reported in Neel *et al.* (1956).

Approximately 300 of the bloods which were sent to the United States for testing were collected by Dr. Marvin D. Solomon in the vicinity of Monrovia; I am indebted to him for permission to include the findings on these bloods in this paper.

The technique used for the determination of sickling in the field was the rapid sodium metabisulfite method. Experimental results with the various techniques fully coincided with Allison's (1954) remarks and recommendations on this subject. The presence of sickle-type hemoglobin (hemoglobin S) in the bloods shipped to the Child Research Center was ascertained by paper electrophoresis and solubility determinations. These techniques are given in detail in Zuelzer, Neel, and Robinson (1956). In order to make the results of the two surveys comparable, those individuals in the paper electrophoresis survey who would have been positive for the sickle cell test were counted as positive without regard to whether they appeared to be homozygous or heterozygous for the sickle cell gene. Thus, the frequency of the sickle cell trait, as used in this paper, includes both heterozygotes and any living homozygotes for the sickle cell gene. However, since recent studies (Lehmann and Raper, 1956) indicate that homozygotes for the sickle cell gene very rarely survive the first few years of life, it is very unlikely that the results of the surveys described in this paper include more than a few homozygotes—if any at all.

Subjects for testing were obtained in several ways. The great majority was obtained either from surveys of Firestone rubber tappers done at the weekly rice issue, from outpatient clinics, or from surveys of schoolchildren. The outpatient clinics and schools were located both around Firestone and in the interior. Strictly speaking, none of these methods of selection provides a random sample of the population. Moreover, some known relatives, and most likely some unknown ones, are included. In an effort to control the number of relatives in the tribal estimates of the frequency of the sickle cell trait, many surveys of small villages in which the majority of the inhabitants were known to be related have been omitted. The number of relatives in the remaining samples is not considered to be large enough to have any appreciable effect on the estimates of the frequency of the sickle cell trait.

Although on many surveys additional information was obtained, tribe, sex, and age were recorded for all subjects. Knowledge of age is exceedingly variable in Liberia, with the majority of adult subjects having no reliable knowledge about their exact age in years. Schoolchildren, however, usually had some information, or in some cases the school authorities knew the approximate ages of their pupils. No special techniques were used for the determination of age, so that the age groups are only approximate with a great deal of overlap between them.

Most of the natives of Liberia speak some English, and when asked their tribe, would respond with one of the tribal names used in this paper. In cases where the tribes of the parents were different and only one name was given in response, it was invariably the tribe of the father. The subjects were not routinely questioned on the tribes of their mother and father; thus it is not known what percentage of the subjects are the result of inter-tribal matings. For the adult subjects, this is most likely a very small percentage. Subjects whose parents were known to belong to different

tribes were included in the tribe of the father. Gene flow is undoubtedly occurring across tribal boundaries in Liberia and these subjects would only be instances of this gene flow.

Except for the peoples who inhabit the southeastern hinterland, the tribes of Liberia are well known. For the areas other than southeastern Liberia the tribal list used in this paper agrees with that of Schwab (1947), except that Schwab includes two additional tribes. These two tribal names were never given to the author in response to questions concerning tribal affiliations. Among authors who have written about the tribes of Liberia, there is considerable disagreement as to the tribes of the interior of southeastern Liberia. This is due mostly to the author's different opinions as to what local group fits the definition of a tribe. In this paper the peoples of this area have been grouped into two large linguistic units, Krahn and Webbo. Although these two groupings, undoubtedly contain several breeding isolates, it is highly unlikely that these isolates differ considerably in the frequency of the sickle cell gene, since the gene is almost completely absent in these peoples.

## RESULTS

Table 1 gives the frequency of the sickle cell trait in the various populations of Liberia. With the exception of the Americo-Liberian, Mandingo, and Fanti, the populations are all indigenous peoples whose ancestors were living in Liberia at the time of European contact. It should be noted, however, that European contact was very late in some areas, as late as the 20th century in the hinterland of southeastern Liberia. The Americo-Liberians are the descendants of slaves who were repatriated

TABLE 1. THE FREQUENCY OF THE SICKLE CELL TRAIT IN THE POPULATIONS OF LIBERIA

| Population | Number examined | Number positive | Sickle cell trait (%) |
|---|---|---|---|
| Americo-Liberian | 106 | 17 | 16.2 |
| Mandingo | 38 | 7 | 18.4 |
| Fanti | 60 | 20 | 33.3 |
| Kissi | 298 | 58 | 19.5 |
| Mende | 77 | 13 | 16.9 |
| Gbandi | 352 | 54 | 15.3 |
| Vai | 93 | 13 | 14.0 |
| Kpelle | 982 | 128 | 13.0 |
| Loma | 511 | 65 | 12.7 |
| Gola | 183 | 22 | 12.0 |
| Belle | 29 | 3 | 10.6 |
| Bassa | 811 | 58 | 7.2 |
| Dei | 53 | 2 | 3.8 |
| Mano | 709 | 15 | 2.1 |
| Gio | 428 | 9 | 2.1 |
| Grebo | 69 | 1 | 1.4 |
| Kru | 148 | 1 | 0.7 |
| Krahn | 154 | 1 | 0.7 |
| Webbo | 77 | 0 | 0.0 |
| Total (Kissi-Webbo) | 4974 | 443 | 8.9 |

from the United States and are mostly scattered along the coast in the larger towns. The Mandingos are rather recent arrivals from French West Africa. Most of them are engaged in commerce and their settlements are located in the larger commercial centers in Liberia, both on the coast and in the interior. The Fantis are fishermen from Ghana. In recent years they have extended their fishing activities to many other countries in West Africa. This sample was obtained from the small Fanti village at Marshall, Liberia.

Prior to combining the data into tribal frequencies, an attempt was made to determine whether sex, age, or method of sampling had any effect on the frequency of the sickle cell trait. Tables 2–4 give the tribal frequencies according to sex, age, and method of sampling, the chi-squares for the differences in frequency for each tribe, and a total chi-square for each table. The five tribes which are shown in tables 3 and 4 are the only ones for which there was a sufficiently large sample for all the age groups and all the methods of sampling to warrant subdivision. The only chi-square which is significant in these tables is that for the frequencies of the sickle cell

TABLE 2. THE FREQUENCY OF THE SICKLE CELL TRAIT IN THE TRIBES OF LIBERIA ACCORDING TO SEX

| Tribe | Males | | | Females | | | Chi-square* |
|---|---|---|---|---|---|---|---|
| | No. Exam. | No. Pos. | Freq. | No. Exam. | No. Pos. | Freq. | |
| Bassa | 617 | 47 | .076 | 194 | 11 | .057 | .843 |
| Kpelle | 841 | 111 | .132 | 141 | 17 | .121 | .124 |
| Loma | 439 | 58 | .132 | 72 | 7 | .097 | .679 |
| Gbandi | 279 | 39 | .140 | 73 | 15 | .206 | 1.923 |
| Kissi | 250 | 50 | .200 | 48 | 8 | .167 | .265 |
| Mano | 564 | 14 | .026 | 145 | 1 | .007 | 1.790 |
| Gio | 375 | 8 | .021 | 53 | 1 | .019 | .014 |
| Gola | 128 | 15 | .117 | 55 | 7 | .127 | .037 |
| Vai | 54 | 8 | .148 | 39 | 5 | .128 | .075 |
| Dei | 40 | 1 | .025 | 13 | 1 | .075 | .729 |
| Mende | 70 | 11 | .157 | 7 | 2 | .286 | .750 |

Total chi-square = 7.229, 11 D.F., $.7 < P < .8$.
* With one degree of freedom P = .05 at $\chi^2 = 3.841$.

TABLE 3. THE FREQUENCY OF THE SICKLE CELL TRAIT IN FIVE LIBERIAN TRIBES ACCORDING TO AGE

| Age Group | Bassa | | Kpelle | | Loma | | Gbandi | | Kissi | |
|---|---|---|---|---|---|---|---|---|---|---|
| | No. Exam. | Freq. | No. Exam. | Freq. | No. Exam. | Freq. | No. Exam. | Freq. | No. Exam. | Freq. |
| 0–5 | 67 | .045 | 35 | .114 | 30 | .100 | 39 | .128 | 16 | .000 |
| 6–10 | 122 | .066 | 80 | .138 | 45 | .089 | 11 | .182 | 16 | .250 |
| 11–15 | 119 | .059 | 77 | .221 | 52 | .135 | 42 | .214 | 19 | .316 |
| 16– | 503 | .080 | 790 | .122 | 384 | .133 | 260 | .146 | 247 | .194 |
| Total | 811 | .072 | 982 | .130 | 511 | .127 | 352 | .153 | 298 | .195 |
| Chi-square* | 1.564 | | 6.256 | | .924 | | 1.581 | | 5.962 | |

Total chi-square = 16.287, 15 D.F., $.3 < P < .5$
* With three degrees of freedom P = .05 at $\chi^2 = 7.815$

TABLE 4. THE FREQUENCY OF THE SICKLE CELL TRAIT IN FIVE LIBERIAN TRIBES ACCORDING TO THE METHOD OF SAMPLING

| Survey | Tribe | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Bassa | | Kpelle | | Loma | | Gbandi | | Kissi | |
| | No. Exam. | Freq. | No. Exam. | Freq. | No. Exam. | Freq. | No. Exam. | Freq. | No. Exam. | Freq. |
| Bloods examined in Detroit (mostly outpatients) | 201 | .085 | 214 | .094 | 159 | .151 | 133 | .135 | 106 | .198 |
| Firestone Rubber tappers | 152 | .072 | 473 | .125 | 168 | .125 | 127 | .165 | 133 | .203 |
| Tappers' dependents | 44 | .000 | 103 | .117 | 45 | .067 | 11 | .182 | 20 | .150 |
| Schoolchildren | 263 | .072 | 189 | .196 | 123 | .114 | 22 | .227 | 19 | .158 |
| Lib. Inst. Trop. Med. Clinic | 150 | .080 | 3 | .000 | 8 | .375 | — | — | 4 | .250 |
| Total | 810 | .073 | 982 | .130 | 503 | .129 | 293 | .157 | 282 | .195 |
| Chi-square* | 4.018 | | 10.566 | | 6.816 | | 1.412† | | 0.572 | |

Total Chi-square = 23.384, 19 D.F., $.2 < P < .3$

* With four degrees of freedom $P = .05$ at $\chi^2 = 9.488$.

† Three degrees of freedom; $.8 > P > .7$.

trait for the Kpelle tribe according to the method of sampling. The relatively high frequency for schoolchildren has made this test significant. Since the schoolchildren frequencies for the other tribes do not seem to be consistently higher than the other frequencies, and since the total chi-square for table 4 is not significant, this is most likely a random deviation. Thus, the schoolchildren have been included in the total Kpelle sample, although there does seem to be some heterogeneity in the sample.

The primary reason for the test shown on table 4 was to determine whether outpatient surveys, involving subjects with some type of illness, would yield results very different in the frequency of the sickle cell trait from those obtained from surveys of complete villages or of workers. In table 4 it can be seen that the results of surveys of outpatients are similar to the results of surveys of tappers and are not consistently higher or lower. Thus, there is no indication from the test that the different methods of sampling would result in different estimates of the tribal frequencies.

Although these statistical tests indicate that the effects of sex, age, and method of sampling are not large enough to influence significantly the tribal frequencies of the sickle cell trait, the possibility still remains that some of the tribes are composed of several breeding isolates which differ significantly from one another in the frequency of the sickle cell trait. Figure 1 shows the distribution of the native tribes of Liberia and their observed frequencies of the sickle cell trait. For southeastern Liberia the frequencies of the sickle cell trait are all very close to zero, so that significant differences between breeding isolates could not exist here. In northwestern Liberia the tribal frequencies are very close to one another, so that differences in frequency within any one tribe would most likely be small. In central Liberia, however, there

FIG. 1. Percentage incidence of the sickle cell trait in the tribes of Liberia.

are several tribes which border on other tribes with very different frequencies of the sickle cell trait. Some attempt was made to sample the sub-areas of these tribes, but the samples were all rather small. No significant differences were found, although there appeared to be some heterogeneity in the Bassa tribe. Further work on this aspect of the distribution is planned. However, even if differences in frequency exist within some of the tribes the general features of the distribution of the sickle cell gene which will now be discussed would not be changed.

## DISCUSSION

From the map it can be seen that there is a northwest-southeast cline in the frequency of the sickle cell trait. With two exceptions the northwestern half of Liberia is occupied by tribes with frequencies ranging from 12 to 20 per cent. The tribes with the highest frequencies of the sickle cell trait are generally toward the interior on the Sierra Leone border, and there is a decrease in the frequency as one proceeds toward the center of Liberia. In the center of the country, the frequency decreases sharply from the Gola and Kpelle to the Dei and Bassa tribes and then from the Bassa to the Kru. In the interior it decreases sharply from the Kpelle to the Mano. In the southeast part of Liberia the frequency then decreases to almost zero per cent,

and in the tribe which is furthest to the southeast and the most isolated, no positives were detected.

Liberia is situated entirely within the West African Tropical Rain Forest, within which the epidemiological picture of malaria varies very little. Young and Johnson's (1949) malaria survey of Liberia showed high parasite rates throughout the country. In fact the highest overall parasite rates occur among the Mano, who have 2 per cent of the sickle cell trait. Thus, the differences in the frequency of the sickle cell trait do not seem to be explained by differences in the intensity of malaria in the areas concerned.

Elsewhere in West Africa, high frequencies of the gene responsible for hemoglobin C tend to be associated with low frequencies of the sickle cell gene and hence in some manner explain these low frequencies. However, the low frequencies of the sickle cell trait found in Liberia cannot be accounted for in this way since the hemoglobin C gene is either very rare or absent in the tribes of Liberia (Neel et al., 1956).

The fact that the frequency of the sickle cell trait in Liberia nowhere approaches the equilibrium value of 40 per cent postulated by Allison does not imply that selection by means of malaria is not an important factor maintaining the high frequencies of the sickle cell gene in Africa, or even in Liberia. It only means that the explanation of the distribution, at least in Liberia, must take into consideration other evolutionary factors. Since the cline in the frequency of the sickle cell trait is smooth and not erratic, and also since there is some correlation between the cline and cultural differences, gene flow, and not gene drift, would seem to be the major factor explaining this cline.

The tribes in the northwestern half of Liberia, who have frequencies ranging from 12 to 20 per cent, speak languages of the West Atlantic or Mande subfamilies of the Niger-Congo linguistic family (Greenberg, 1955). Within this group language affiliation does not seem to be correlated with the frequency of the sickle cell trait, the cline being gradual throughout regardless of language. The Kissi, who have the highest frequency in this area, and the Gola, who have the lowest, speak West Atlantic languages. The Mende, Gbandi, Loma, and Kpelle speak Southwest Mande-fu languages, while the Vai speak a Mande-tan language. The same is also true in Sierra Leone, where the Timne, who speak a West Atlantic language, have 29 per cent of the sickle cell trait (Allison, 1956), and the Mende, who speak a Mande language, have 30 per cent (Allison, 1956; Rose and Suliman, 1955). These frequencies are higher than any encountered in Liberia, and it is also interesting to note that the difference in frequency between the Mende in Liberia and the Mende in Sierra Leone is greater than the difference between the Timne and the Mende in Sierra Leone. Thus, the Mende tribe seems to contain several breeding isolates which differ significantly in the frequency of the sickle cell trait.

Although the cline in the frequency of the sickle cell trait in northwest Liberia and Sierra Leone is not correlated with language, the sharp decrease in the frequency which occurs in central Liberia is related to linguistic differences. The Dei, Belle, and Bassa, who have lower frequencies, are the northernmost speakers of Kru languages. Neither the Dei nor the Belle frequency is significantly different from that

of the Bassa, although with larger samples from these tribes they might very likely become so. Nevertheless, the decrease in the frequency of the sickle cell trait from the Gola and Kpelle on the north to the Dei and Bassa on the south is related to linguistic differences. Similarly, in the interior of central Liberia, the significant decrease which occurs on the Kpelle-Mano border is related to linguistic differences. Mano and Gio, which are Northeast Mande-fu languages, were previously thought to be closely related to Kpelle and other Mande languages whose speakers have high frequencies of the sickle cell trait, but Prost (1953) has recently shown that they are related to the Mande languages of the Ivory Coast and not to their Mande neighbors in Liberia.

Recent unpublished investigations by Binson, Neel, and Zuelzer in the Ivory Coast to the east of Liberia indicate a similar cline in the frequency of the sickle cell trait. Starting with the high frequencies of more than 20 per cent in Ghana, which is the east of the Ivory Coast, there is a gradual decrease in the frequency up to the central Ivory Coast where there is an abrupt decline on the border of the Akan peoples on the east and the Kru and Lagoon peoples on the west. The frequency then decreases to almost zero per cent among the Kru peoples of the western Ivory Coast. Thus, in eastern Liberia and the western Ivory Coast, there is a "pocket" of tribes with low frequencies of the sickle cell trait, who are surrounded by tribes with high frequencies. A similar "pocket" has also been found recently by Leite and Ré (1955) in Portuguese Guinea. Since the tribes with low frequencies in both these pockets are the more primitive and isolated tribes of West Africa, the sickle cell gene appears to have been introduced into the western part of West Africa and is still spreading to the more backward peoples.

The almost complete absence of the sickle cell trait among many of the Kru peoples is another surprising finding of this investigation. Although no anthropometric studies were done, the author would agree with most observers who have written about Liberia, that the Kru peoples are the most Negroid of the tribes of Liberia. Holas (1952, p. 339), on the basis of anthropometric measurements, has called the Krahn, who are one of the Kru peoples of the interior, the "true Negro". Johnston (1910) regards the Kru as the archtype of the race he calls the "Forest Negro", and Hooton (1947, p. 622) says "The purest type of African Negro, called by Sir Harry Johnston, the 'Forest Negro', is found along the coastal regions of West Africa . . . .". It thus seems that the sickle cell gene, although long considered a Negro characteristic, is absent in the "purest" representatives of the Negro race.

SUMMARY

Data are presented on the distribution of the sickle cell gene in Liberia. These data indicate a northwest-southeast cline in the frequency of the sickle cell trait. In the extreme northwest on the Sierra Leone border there is 20 per cent of the sickle cell trait, and the frequency decreases gradually to about 12 per cent in central Liberia. There is then an abrupt decline to 4 to 7 per cent among the coastal tribes and to 2 per cent among the tribes of the interior, and then a more gradual decrease to almost zero per cent in southeastern Liberia. The evidence seems to indicate that this cline is due to the recent advance of the sickle cell gene through Liberia.

ACKNOWLEDGMENTS

The research on which this paper is based was carried out while the author was a Public Health Service Predoctoral Research Fellow of the National Heart Institute; the findings were submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Michigan.

The author is indebted to Dr. Max J. Miller both for making the facilities of the Liberian Institute of the American Foundation for Tropical Medicine, Inc. available to him and for a great deal of helpful assistance in connection with the field work. He is also indebted to Drs. James V. Neel and James N. Spuhler for many helpful suggestions during the course of this study and the preparation of the manuscript.

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# A Genetic Study of Carcinoma of The Large Intestine[1]

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## INTRODUCTION

IT IS NOW WELL ESTABLISHED that multiple polyposis is a genetic disease, with a dominant mode of inheritance. A review of the subject has been presented by Dukes (1952), and a recent study has been carried out by Reed and Neel (1955). This condition is characterized by the presence of a large number of adenomatous polyps of the colon and rectum. Since the age of onset may be during the first or second decade of life and each polyp appears to be a potentially malignant lesion (Lahey and Swinton, 1952), individuals with the disease often die at an early age from carcinoma of the colon or rectum. Reed and Neel (1955) estimate that in the State of Michigan, the minimum frequency at birth of individuals with the gene for multiple polyposis is about 1 in 8,300. They further estimate that the mutation rate lies somewhere within the range $1\text{–}3 \times 10^{-6}$/gene/generation.

Even though multiple polyposis is a rare disease, the occurrence of a single polyp or a few polyps (occasional discrete polyps) in the colon or rectum is a relatively common condition. Routine sigmoidoscopic examinations carried out in various clinics in the United States have shown that approximately five per cent of all individuals over 35 years of age have one or more polyps of the rectum or sigmoid (Rider, Kirsner, Moeller, and Palmer, 1954; Swinton, 1954). Multiple polyposis is easily distinguished from the latter condition. It is a matter of hundreds or even thousands of polyps versus one or a few.

In 1955, Woolf, Richards, and Gardner presented evidence that in some cases the occurrence of occasional discrete polyps may be influenced by genetic background. Fifty-five adult members belonging to the same generation of a large Utah kindred with a high frequency of carcinoma of the large intestine were given sigmoidoscopic examinations. Twenty-five (45 per cent) were shown to have one or more histologically confirmed polyps. This is a much higher frequency than is expected in the general population. As a further control, similar examinations were given to twenty-one of their spouses. One spouse was shown to possess a questionable polyp, while all other spouses were normal. Twelve to eighteen months after the initial examinations were given, re-examinations were given to some of the members (Richards and Woolf, 1956). Over half the individuals positive for polyps at the first sigmoidoscopic examination were shown to have one or more new polyps at the time of the second examination. Some individuals negative at the first examination were positive at the

Received August 12, 1957.

[1] This study was supported by a field investigation grant from the National Cancer Institute, of the National Institutes of Health, Public Health Service.

second. The distribution and frequency of positive individuals in the kindred was suggestive of dominant inheritance.

The above observations suggested that carcinoma of the large intestine could have a familial tendency in some cases due to a genetic mechanism other than that associated with multiple polyposis. In order to gain more information on this problem, a propositus study was carried out. The objective was to compare the frequency of deaths due to carcinoma of the large intestine, and to other types of cancer, among the relatives of individuals who died from large bowel cancer not associated with multiple polyposis, with the frequency in a control sample. It was proposed that if another genetic mechanism were operating, such as through the production of occasional discrete polyps, it would be expected that the close relatives would show an increased frequency of deaths due to the disease as compared with an adequate sample taken from the general population.

## PROCEDURE AND RESULTS

A diversity of methods has been employed in propositus studies designed to investigate the importance of heredity as an etiological factor in human cancer. Many of these have been reviewed in Jacobsen (1946) and Woolf (1955). The method employed in the present study was used by the present author (Woolf, 1956) in a propositus study on carcinoma of the stomach. The advantages and disadvantages of the method were discussed in that publication, and will not be repeated here.

Propositi for the present study were picked from death certificates for individuals who died in the state of Utah between the years 1931 and 1951. Individuals were selected only when there was substantial evidence on the death certificate, as reported by the attending physician, that carcinoma of the large intestine was actually present. Selection was made for death certificates for white persons born or raised in Utah or in the surrounding intermountain region. Certificates were not included if there was any indication that multiple polyposis had been present.

Upon obtaining the names of a large series of individuals who died from carcinoma of the large intestine, the next step was to take the names to the archives of the Latter-Day Saints (Mormon) Genealogical Society in Salt Lake City, Utah. On file in this office are family group records which are useful for studies of this kind. A survey was made of the records to see if information was available for the families (parents and sibs) of the individuals who died of carcinoma of the large intestine. A family group record was available for approximately one-fourth to one-fifth of the names selected from the death certificates. Upon obtaining the genealogical data the next procedure was to return to the Utah State Division of Vital Statistics. With the aid of the cross index file for death certificates, a careful search was made for the names of the parents and sibs of the individuals who died from carcinoma of the large intestine. Death certificates have been filed in this office since 1905. If a relative died in Utah since that time the certificate was usually found. However, the certificate was not found if the relative died before 1905, had a change in name, died in some other state, or was still alive. The study was concerned only with those relatives who had died in Utah since 1905. As soon as a certificate was located for a relative, notes were made of the cause and date of death and of the age at death.

The study was continued until histories were obtained for 242 families for which at least one death certificate in addition to that of the propositus was seen. One family was not included because the death certificate of one relative of the propositus gave evidence of multiple polyposis.

The control material used for comparison consisted of white individuals of the same sex, who died the same year in the same county and at approximately the same age as each of the deceased relatives of the propositi. Death certificates in Utah are filed by date of death and county, and bound into books. When a death certificate was found for a relative of a propositus, it became a matter of turning the pages of the book until the first qualified control was found. To be considered as approximately the same age as a relative of the propositus a control's age had to be within minus three and plus five years of the relative's age.

The death certificates for 145 fathers, 142 mothers, 309 brothers, and 167 sisters were observed, giving a total of 763. Twenty-six of these had cancer of the large intestine as compared with 8 in the controls. Chi square computed from a 2 x 2 table is equal to 8.7 ( $P < .01$). The number of deaths due to large bowel cancer was increased over the controls in all four classes of relatives.

Eighty-one deaths from cancer other than of the large intestine were observed

TABLE 1. COMPARISON OF THE NUMBER OF DEATHS DUE TO CANCER OF THE LARGE INTESTINE AND OTHER TYPES OF CANCER IN THE FAMILIES OF THE PROPOSITI AND AMONG THE CONTROLS

| Relation | Death Certificates Observed | Deaths Due to Cancer of the Large Intestine | | Deaths Due to Other Types of Cancer | |
|---|---|---|---|---|---|
| | | Families of the Propositi | Controls | Families of the Propositi | Controls |
| Father | 145 | 4 | 1 | 12 | 11 |
| Mother | 142 | 6 | 0 | 8 | 20 |
| Brother | 309 | 9 | 6 | 45 | 33 |
| Sister | 167 | 7 | 1 | 16 | 19 |
| Total | 763 | 26 | 8 | 81 | 83 |
| | | $\chi^2 = 8.7^*$ $P < .01$ | | $\chi^2 = 0.007^*$ $.90 < P < .95$ | |

* Chi square based on totals (one degree of freedom).

TABLE 2. COMPARISON OF THE NUMBER OF DEATHS DUE TO CANCER IN THE FAMILIES OF THE PROPOSITI AND AMONG THE CONTROLS

| Type of Cancer | Relatives of the Propositi | Controls |
|---|---|---|
| Large Intestine | 26 | 8 |
| Stomach | 26 | 33 |
| Breast | 2 | 6 |
| Small Intestine | 0 | 1 |
| Uterus or Ovaries | 8 | 5 |
| Liver or Pancreas, or Gall Bladder | 9 | 9 |
| Prostate | 9 | 6 |
| Lung | 3 | 3 |
| Other Types | 24 | 19 |

TABLE 3. DISTRIBUTION OF DEATHS DUE TO CANCER OF THE LARGE INTESTINE IN THE FAMILIES OF THE PROPOSITI

| Number of deaths due to Cancer of the Large Intestine | Observed Number of Families |
|---|---|
| 0 | 221 |
| 1 | 17 |
| 2 | 3 |
| 3 | 1 |
| 4 or more | 0 |
| Total | 242 |

among the relatives and 83 among the controls. The difference is clearly not significant ($.90 < P < .95$). The results are summarized in table 1.

The different types of cancer deaths occurring among the relatives of the propositi and controls are shown in table 2. It is observed that with the exception of cancer of the large intestine, there is good agreement between the relatives and controls concerning the frequency of the various types of cancer deaths.

The distribution of deaths due to cancer of the large intestine in the families of the propositi is shown in table 3.

## DISCUSSION

Even though an attempt was made to select propositi who were free of multiple polyposis, due to the inherent nature of death certificates, there is no certainty that this was achieved in all cases. Therefore, it is important to consider whether the occurrence of this disease in some of the families could be contributing to the significant difference in the frequency of deaths due to carcinoma of the large intestine between the relatives and controls. This possibility was investigated by examining all available evidence from the records of autopsies, operations, x-rays, and sigmoidoscopic examinations of the 26 relatives who died of cancer of the large bowel and of the propositi to whom they were related.

Medical records definitely excluding multiple polyposis were available for eight of the 26 relatives. For the remaining 18 relatives, medical records were either missing or else incomplete with regard to the predisposing condition of the bowel. Seven of these 18 relatives were removed from the possible multiple polyposis group because of the conclusive evidence that the related propositi did not have the disease; thus it was concluded that the gene for multiple polyposis was not segregating in these families. This leaves 11 relatives for whom information was not available. The ages at time of death for these ranged from 60 to 82 years with a mean of 70.7 years. The ages at time of death for the related propositi of these 11 individuals ranged from 63 to 80 years with a mean of 72.4 years. These ages at death are much higher than expected for patients with multiple polyposis. The average age at death in a large series studied by Dukes (1952) in England was 41.6 years, while in the general population of England and Wales, the average age at death from rectal cancer is 67 years, This age difference has also been reported by Reed and Neel (1955) for data collected in Michigan. The above evidence suggests strongly that the results of this

study are not attributable to the segregation of the gene for multiple polyposis in some of the families. Hence the data are compatible with the hypothesis that a genetic mechanism exists for carcinoma of the large intestine which is independent of the one associated with multiple polyposis.

On the basis of experimental work with mice, Heston (1952) has suggested that cancer occurs in mice when the combined action of genetic and non-genetic components, which are probably additive in nature, surpasses a certain physiological threshold. It seems likely that a similar situation exists for human cancer, although at present little is known of the nature and relative importance of these genetic and non-genetic components. Even though, in the present study, the number of deaths, among the relatives of the propositi, due to cancer of the large bowel is significantly greater than the number among the controls, it is not large enough to suggest that heredity is of greater etiological importance than some other unknown non-genetic factors. Therefore the genetic component encountered here appears to have about the same magnitude of importance as has been shown for carcinoma of the stomach and breast (see Woolf, 1955, 1956).

Of interest is the observation that with the exception of carcinoma of the large intestine, the number of deaths due to other types of cancer among the relatives of the propositi was not increased over that in the controls. This organ-specificity of the genetic component is similar to that observed for stomach cancer (Videbaek and Mosbech, 1954; Woolf, 1955, 1956) and breast cancer (Penrose, MacKenzie, and Karn, 1948; Woolf, 1955). These observations illustrate further the independence of the genetic mechanisms leading to the occurrence of neoplasia.

On the basis of the observations of Woolf, Richards and Gardner (1955), and Richards and Woolf (1956), it is speculated, as noted earlier, that one possible method by which the genetic component encountered in this study predisposes to the malignant condition is by the production of occasional discrete polyps in the colon or rectum. Genetic factors predisposing to these polyps in some families could account for the findings of this study.

## SUMMARY

A propositus study was carried out to test the hypothesis that a genetic component, independent of the one associated with the familial disease, multiple polyposis, exists for cancer of the large intestine. The results of this study are compatible with this hypothesis.

Propositi were obtained from death certificates for the state of Utah and family histories from the records of the Latter-Day Saints Genealogical Society in Salt Lake City, Utah. Causes of death for the relatives (sibs and parents) were determined from death certificates for the state of Utah. The control material was also obtained from death certificates.

## ACKNOWLEDGMENTS

Appreciation is extended to Mr. John W. Wright, Director of the Division of Vital Statistics for the State of Utah, and Mr. L. Garrett Myers of the L. D. S.

Genealogical Society in Salt Lake City, Utah, for permission to use their facilities and records in carrying out this study.

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# Hereditary Multiple Leiomyoma of The Skin

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LEIOMYOMATA OF THE SKIN are tumors which consist of spindle-shaped, smooth, muscle fibers, running in various directions and embedded in a more or less abundant stroma of connective tissue. The solitary tumors are thought to derive from blood vessel walls. In contrast, the multiple type is considered to take its genesis from the arrectores pilorum. Sexes are equally affected. The onset for 50 per cent of the cases is before the age of 20 years. The ages of the patients at the time of examination range from 18–60 with an average age of 35. The tumors very slowly increase in size and number from the time they are first noticed. Virchow (1854), the author of the word myoma, described the first example of leiomyoma of the skin which he had observed in a 32-year-old male with multiple perimammillary tumors.

In a search of the literature Stout (1938) found 80 cases that had been reported and he added 15 more. To the present time approximately 100 reports have been recorded in the literature. Textbooks in dermatology state that no known etiological factor can cause multiple leiomyoma of the skin (Sutton, 1956), and that "There is no evidence of any hereditary factor" (Eller and Eller, 1951) associated with this condition. In the present study we find that a dominant gene not only can be the cause of multiple leiomyoma of the skin, but that it may be the only known cause for this condition.

The pedigree chart in our study was made when a patient of Italian descent was sent to Tulane Medical School by Dr. J. W. Burks because of the interesting features presented. This patient (the propositus in our study) was the first that had been seen with this particular tumor at the Tulane Medical Center by the present staff. In subsequent inquiries among dermatologists this patient seems to be the first case recognized in roughly 500,000 patient visits. The present study was undertaken to explain the heredity and to compare the pathology of the lesion with recorded cases in the literature, to learn whether the pathology differs from cases not known to be hereditary.

The pedigree of the propositus is shown in figure 1. The information for this chart was obtained from the propositus and from a 45-year-old unaffected sister (no. 18). The propositus is the mother of three children, ages 10, 7, and 5, who are reported by the mother to be unaffected. It should be emphasized that small inconspicuous lesions could easily escape recognition by the mother even though they were present at these ages. This family lives 150 miles from New Orleans and only the mother was available for observation. The 36-year-old brother (no. 19) of our propositus has three children, ages 8, 6, and 2. This family lives in New Jersey and was also not

Received August 12, 1957.

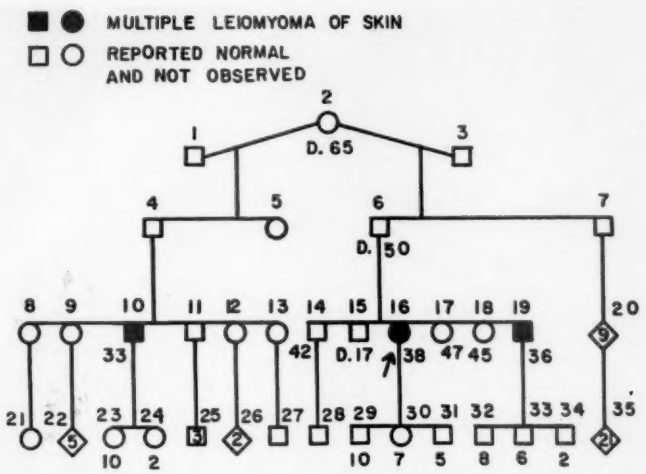


FIG. 1. Pedigree showing occurrence of multiple leiomyomata of the skin in a family of Italian descent. Reference numbers of individuals are above the symbols; numbers within symbols are numbers of individuals, if more than one, represented by a single symbol. The number beneath the symbol is the age or age at death.

available for observation. The 33-year-old half first cousin (no. 10), who is also affected, lives with his family several hundred miles in another direction from New Orleans and was also unavailable for examination. However, through correspondence with physicians of the affected members of this family who lived outside the state, biopsies were secured from which the diagnosis of multiple leiomyoma was confirmed. It should be emphasized that the diagnosis of each affected person on our chart has been confirmed by biopsy.

Within the limitations of the information available, two possible modes of inheritance are apparent. The more probable of these two possibilities is transmission by an autosomal dominant gene. The distribution of cases can be explained in two ways. First, the gene could be highly penetrant causing at least a few inconspicuous lesions in some of the individuals, numbers 2, 4, and 6 in particular, who were reported by the propositus to be unaffected. It is regrettable that an examination of these individuals was not possible. Second, an autosomal gene with reduced penetrance could also explain this distribution of affected individuals within the family if it be assumed that no lesions would be observed in any of the individuals who were not examined and if it be further assumed that all these individuals have lived through the age range of expected onset. Although the numbers are too small for statistical analysis, a dominant gene would be expected to be present in individuals 2, 4, and 6 and in half of the 12 offspring of individuals 4 and 6. Evidence of effects from the gene in 3 of these 9 expected carriers of the gene (six of the 12 offspring plus individuals 2, 4, and 6) would make the penetrance of the gene approximately 33 per cent.

The second possible genetic explanation for the occurrence of multiple leiomyoma

FIG. 2. Photograph of multiple leiomyomata on the upper arm extensor surface of the left arm of the propositus represented in Figure 1.

of the skin in this family is the presence of an autosomal recessive gene with relatively high penetrance. On this basis individuals 2, 4, and 6 would be presumably heterozygous and thus not expected to be affected. For individuals 10, 16, and 19 to be homozygous and affected, the spouses of 4 and 6 would each have to be heterozygous. These spouses seem to have no lines of descent from an ancestor common to individual 2 so the probability that each spouse would be a carrier is very small; to be specific, the probability that these spouses would be carriers is approximately the frequency of encountering at random a heterozygous carrier in the general population. The fact that so few cases of multiple leiomyoma of the skin have been observed could be due to the infrequency of carriers or to the infrequent reporting of cases. Since no accurate estimate is available for the incidence of leiomyoma of the skin, no estimation can be made for the frequency of heterozygous carriers in the population, if the mode of inheritance is assumed to be a recessive. However, it would seem that the presence of an autosomal dominant gene is a much more probable explanation for the occurrence of these cases in this family than the presence of an autosomal recessive gene.

Figure 2 is a photograph of the multiple leiomyomata on the arm of our propositus (no. 16) who also had patches of papules over the extensor surface of the right arm and left thigh. The individual lesions varied from 2 to 12 mm in diameter, raised up to 5 mm. The color ranged from pink, to red, to brown. The consistency of a papule was soft to moderately firm. There was some local tenderness with moderate pressure.

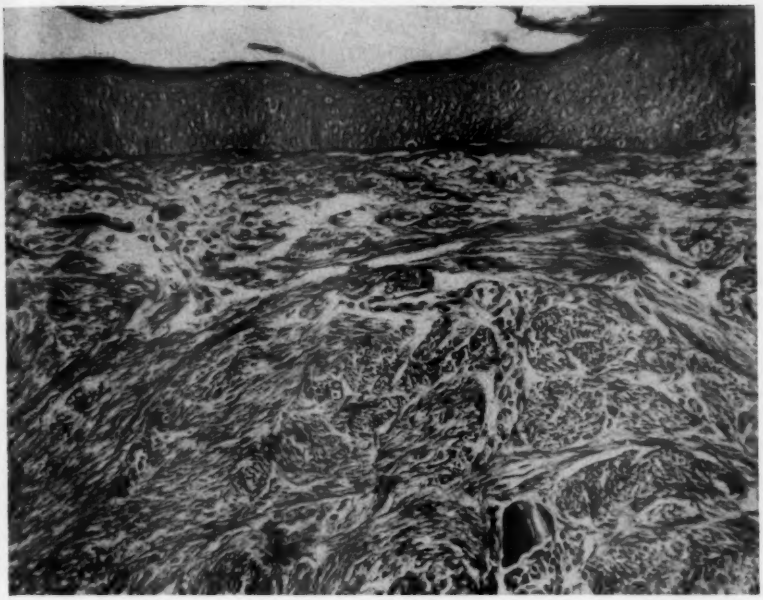

FIG. 3. Microscopic section of leiomyoma of the skin from individual 16 in figure 1.

Examination of routine hemotoxylin-eosin sections of a single papule (see figure 3) revealed the dermis filled with irregularly outlined but well defined masses of pink staining bundles of fibers interwoven in many directions, so that some fibers were cut crosswise, some longitudinally. Basophilic nuclei were noted in the cross-sectioned areas to be within the fibers. Small numbers of round cells, lymphocytes and histiocytes, were present in trabeculae traversing the tumor area. With Masson's trichrome stain, the tumor tissue stained pink, confirming the muscular nature, in contrast to the blue stain of collagen. The clinical picture and histopathology of the tumors observed in this family differ in no way from the descriptions typically recorded in the literature for multiple leiomyoma of the skin.

No publication on multiple leiomyoma of the skin in which more than one case within a family had been verified by biopsy was found in a review of the literature. However, precedence was found for the occurrence of affected relatives with unconfirmed diagnosis. Hagena (1925) observed patches of tumors in a maternal uncle of his male patient. The tumors in this uncle appeared in no way different from those observed in his patient. Since the uncle would not consent to a biopsy, this author did not have the proof necessary to claim more than one affected case in this family. Garibaldi (1925) observed tumors in the father of his male patient and he was told that the paternal grandfather also had a similar type of tumor. Diagnosis by biopsy in this family likewise was limited to the propositus. At a recent national meeting of dermatologists informal inquiry was made about the occurrence of this disease. One dermatologist in the group who had seen a patient with this condition was Dr. H.

E. Michelson, Professor of Dermatology at the University of Minnesota. Dr. Michelson (1957) stated that the mother of his female patient also had multiple leiomyoma of the skin. Thus, we have found that although confirmed diagnosis of secondary cases in a family have not been reported previously, other affected relatives have probably occurred in at least three families. In each of the instances cited in which others in the family were probably affected, the mode of transmission was consistent with autosomal dominant inheritance. If selection of these secondary cases for citation was not dependent upon high expressivity, there would be reason to believe the penetrance of this dominant gene is greater than the value estimated from our own data.

The fact that obviously affected relatives of patients have been considered by authors of textbooks to be unaffected because the diagnosis was not confirmed by biopsy has led to the generalization that heredity is not a cause of this disease. It is as serious an error to state that unexamined relatives are normal when they are in reality affected as it is to state that unexamined relatives are affected when they are not affected. It would seem that clinicians should be as careful about avoiding false negative diagnoses among relatives of patients as they are in avoiding false positive diagnoses. Otherwise, a body of literature accumulates which gives the impression that heredity is not a factor when actually it may be the only factor that can account for the pathology. A record should be made about age, sex and whether or not an examination was made before a relative is recorded as normal.

## SUMMARY

The rarity of leiomyoma of the skin is shown by the fact that only approximately 100 instances have been recorded in the literature in over a century and by the fact that the propositus in our family was the only such case seen in roughly 500,000 patient visits. Heredity is believed to be the best explanation and at present the only explanation for this condition in the two siblings and a half first cousin here reported. Previous evidence that a gene could be responsible for this lesion was insufficient probably because affected relatives either were not examined or the diagnosis was not confirmed by biopsy. New mutations might well account for many of the isolated cases reported in the literature. Reduced penetrance or failure of the observer to note inconspicuous lesions among reportedly normal relatives could account for the remaining isolated cases. If all adult members of the present family reported by the informants as unaffected really carried no lesions, the penetrance of the autosomal dominant gene for this condition would be about 33 per cent.

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# The Detection in the Heterozygote of the Metabolic Effect of the Recessive Gene for Phenylketonuria*

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PHENYLALANINE TOLERANCE TESTS will distinguish the phenotype of most individuals heterozygous for the phenylketonuria gene. Hsia, Driscoll, Troll and Knox (1956) found that the plasma levels after a given dose of phenylalanine were higher and more sustained in nineteen known heterozygotes than in nineteen control individuals, presumably because the heterozygotes had less than the normal amount of the gene-controlled phenylalanine hydroxylase. This enzyme is almost completely absent in the homozygous condition (Jervis, 1953; Udenfriend, 1953). It appeared possible that a similar distinction between normal and heterozygous individuals might also be made without the administration of phenylalanine. The approximate basal plasma levels of phenylalanine before its administration had been determined routinely in the 1956 study as a precaution against the use of individuals with already elevated levels. The basal values observed were at the limit of sensitivity of the method used and were consequently subject to large errors, but there appeared to be a difference between the mean basal levels recorded for the groups of control and heterozygous individuals which was statistically significant and which persisted when more individuals were studied (Hsia and Driscoll, 1956).

The present paper describes a sensitive and accurate method for the determination of the fasting levels of plasma phenylalanine and a reinvestigation of this apparent difference between control and heterozygous individuals. The new analyses confirm the existence of a difference in phenylalanine levels between groups of normal and heterozygous individuals. With the more precise analysis there is relatively little scatter of the values in each group and little overlap between the groups, so that the majority of individuals may be correctly assigned to one group or the other by this determination alone.

## SUBJECTS

*Heterozygotes for Phenylketonuria:* Nineteen adults were parents of known phenylketonuric patients. Nine of these parents had been studied previously with phenylalanine tolerance tests. Four presumed heterozygotes were added to this group of parents. Three of these were teen-age siblings of phenylketonuric patients. The fourth was an adult whose family, although studied extensively, included no cases of

Received August 7, 1957.

* This investigation was supported by Grant A567 from the National Institute of Arthritis and Metabolic Diseases, United States Public Health Service, and by the United States Atomic Energy Commission contract No. AT(30-1)-901 with the New England Deaconess Hospital.



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phenylketonuria. These four had been diagnosed as heterozygotes for phenylketonuria by the phenylalanine tolerance test. One or more samples of blood were drawn from each of these 23 individuals after an overnight fast.

*Control Individuals:* Blood samples, usually taken two to four hours after the last meal, were obtained from 26 adult individuals when they made donations at the local blood bank. Seven additional adults, members of the laboratory personnel, were studied after an overnight fast. The length of the elapsed time interval after eating appeared to have no effect on the results, since there was no significant difference in phenylalanine levels between the two groups of controls studied.

## METHODS

L-phenylalanine was determined specifically with the phenylalanine decarboxylase of *Streptococcus faecalis* R (McGilvery and Cohen, 1948), followed by colorimetric determination of the phenylethylamine formed with a modification of the method of Udenfriend and Cooper (1953).

*S. faecalis* R were grown for 18 to 24 hours at 37° C. without aeration in 15 L. of autoclaved medium containing 3 per cent casein digest, 0.5 per cent yeast extract (Difco), 1 per cent glucose, (autoclaved separately), 72 g. of $NaH_2PO_4 \cdot H_2O$, and 18 g. of $Na_2HPO_4$. The yield was determined by the buffer action of phosphate in maintaining the pH between 5 and 6. With this amount of phosphate about 6 g. dry weight of cells were produced before the pH of the medium fell to about 4.5 and growth stopped. The bacteria were harvested with a Sharples Supercentrifuge and washed twice with water and twice with 10 volumes of ice-cold acetone in an ordinary centrifuge. The dry powder was stored in a tightly closed bottle at 4° C. It remained usable for at least six months.

Plasma from the oxalated blood samples was deproteinized by the addition of 0.1 volume of 1 N acetic acid, heating 3 min. at 100°, followed by the addition of 0.1 volume of approximately 0.5 N NaOH. The mixture had a final pH of 5.4–5.6 at which maximal precipitation occurred, and at which the filtrate was poised for the subsequent enzyme reaction. The plasmas were separated the same day the blood was drawn and were made into filtrates the same day or the next. Filtrates could be kept frozen for several weeks before the level of phenylalanine diminished significantly.

To obtain the necessary sensitivity and accuracy of the phenylalanine determinations, and to avoid certain difficulties inherent in the original method, the following procedure was used. The decarboxylation reaction was carried out in 15 ml. glass-stoppered centrifuge tubes containing 0.025 to 0.075 μmoles of L-phenylalanine, present in 0.5 ml. of plasma filtrate or added from a fresh 1:100 dilution of 0.05 M stock standard solution. 0.4 ml. of 0.7 M sodium citrate buffer, pH 5.5, 12 mg. *S. faecalis* R powder freshly suspended in the buffer, and water were added to a total volume of 1.0 ml. per tube. Duplicate blanks without phenylalanine, and duplicate standards with at least two concentrations of phenylalanine were run with each set of determinations. The bacterial suspension was added last to the reaction mixture after it was warmed to 37° C. The tubes were incubated with shaking for exactly three hours at 37° C. during which time the decarboxylation followed first order

kinetics and went substantially to completion. The reaction was stopped by the addition of 0.15 ml. 10 N NaOH. 2.3 ml. of chloroform containing 3.3 per cent by volume of iso-amyl alcohol was added from a syringe pipette and the mixture shaken for 5 min. to extract the phenylethylamine. The aqueous supernatant solution and bacteria were aspirated off after centrifugation at 2,500 rpm for 3 min. A 1.5 ml. aliquot of the chloroform layer was transferred to optically matched glass-stoppered 9 mm. (O.D.) tubes. After adding 0.15 ml. of a fresh 1:1 mixture of saturated boric acid and 0.5 per cent methyl orange (the latter solution exhaustively extracted with the chloroform-iso-amyl alcohol solution to remove impurities), the tubes were shaken for 2 min. and recentrifuged. The tubes were dipped into warm water (45–50° C.) to remove turbidity, wiped and read at 430 mμ in a Coleman Jr. spectrophotometer. The optical density was proportional to the phenylalanine concentration and to the amount of filtrate used up to 0.1 μmole of phenylalanine (optical density about 0.3). In about half the values reported duplicate determinations were done and the mean of the two was used.

## RESULTS

*Accuracy of the Method:* As an indication of the accuracy of the method of analysis, the agreement between duplicate analyses was studied statistically. Twenty-two samples (from thirteen controls and nine heterozygotes) were determined in duplicate. The mean difference between duplicates was 0.0065 μmoles/ml. (0.0059 and 0.0069 for the heterozygote and control groups, respectively). The standard deviation of a single analysis was ±0.0059 μmoles/ml., (standard deviation

$$= \sqrt{\frac{\Sigma(\text{difference between duplicates})^2}{2 \times (\text{number of differences})}}$$

and the standard error of the mean of duplicate analyses was ±0.0042 μmoles (standard error of the mean of duplicate analyses $= \frac{\text{standard deviation}}{\sqrt{2}}$. At a confidence level of 95 per cent, therefore, the true mean would be expected to be within ±0.008 μmoles of the observed value. In an equal number of subsequent determinations with the same method the variation between duplicates was about half as great (unpublished observations of Barbara Rosen).

The absolute accuracy of this method was judged by comparison of the levels found for normal plasma by it and by the best of the other methods. Ion-exchange chromatography is generally accepted as the most reliable method for the determination of plasma phenylalanine, and the values obtained with it are substantially lower than those determined by microbiological methods or by the Kapeller-Adler chemical method. The levels of plasma phenylalanine determined by ion-exchange chromatography have been reported for about ten individuals (Stein, Bearn and Moore, 1954; Evered, 1956) all of which fell within the range of 0.040 and 0.064 μmoles/ml. (0.67–1.05 mg. per cent). Thirty-two of the values in control individuals determined in the present study with the decarboxylase method ranged from 0.045 to 0.089 μmoles/ml., with a mean of 0.068 μmoles/ml. The present method therefore

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TABLE 1. PLASMA PHENYLALANINE LEVELS IN INDIVIDUALS AT TWO DIFFERENT TIMES

| | Time Between Samples | First | Second |
|---|---|---|---|
| 1. | 11 months | .101* μmoles/ml. | .099* μmoles/ml. |
| 2. | 2 months | .089 | .087 |
| 3. | 2 months | .096 | .115 |
| 4. | 4 days | .066 | .070* |
| 5. | 11 months | .085 | .066* |

* Mean of duplicate determinations.

TABLE 2. MEAN PLASMA PHENYLALANINE LEVELS

| | Mean | Standard Deviation |
|---|---|---|
| Controls (32)* | 0.068 μmoles/ml. | ±.010 |
| Heterozygotes (22)** | 0.103 | ±.016 |

* One control whose level was 0.118 μmoles/ml. has been excluded from the mean.
** One teen-age individual whose level was .205 has been excluded from the mean.

appears to yield substantially the same absolute values as ion-exchange chromatography.

Determinations on two samples of blood drawn on different days were done in five individuals. All were fasted overnight before each test. The results, given in table 1, show that the levels were remarkably constant for periods as long as eleven months between samples. The factors, possibly physiological, which might be responsible for the variations which did occur in plasma phenylalanine levels were not identified. The changes, in general, would not be sufficient to interfere with the correct classification of an individual as a control or a heterozygote.

*Values in Individuals:* The means and standard deviations of the groups of 32 controls (excluding one who was possibly a heterozygote) and 22 heterozygotes (excluding the extremely high value of a teen-age girl) are given in table 2. There is a highly significant difference between the two groups ($t = 10$ for the difference between means, with 52 degrees of freedom). The observed distribution of all the levels at intervals of 0.005 μmoles/ml., which reflects the reproducibility of the measurements over the most important parts of the range, is shown graphically in figure 1. With one exception, the distributions of the two groups overlapped only in the zone from 0.075 to 0.089 μmoles/ml., which is about what would be predicted from the standard deviations of the means. Eleven out of the total of 56 individuals, or 20 per cent, fell within this zone of overlap. The levels for 80 per cent of the individuals fell either below or above this zone of overlap and could be clearly assigned either to the control or the heterozygote group, respectively. The classification of five of the 23 heterozygotes (22 per cent) would be in doubt because of this overlap if they were found in the normal population.

The presumed heterozygotes, one adult without a family history of phenylketonuria and three teen-age siblings of phenylketonurics, were all clearly in the heterozygote range in the present test. Their levels were 0.098, 0.120, 0.136 and 0.205 μmoles/ml. respectively. The hereditary nature of the high phenylalanine level, shown by the fact that both parents of each of these three siblings also had high levels, increased the likelihood that the presumed heterozygotes were in fact heterozygous for the phenyl-

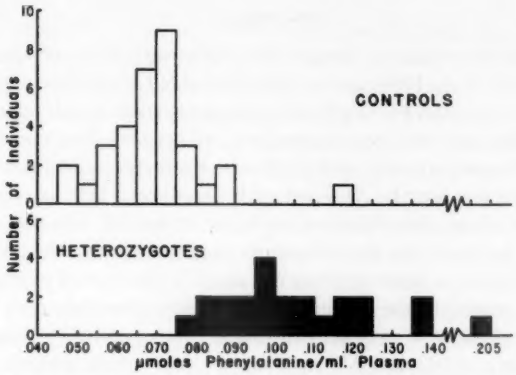


FIG. 1. Distributions of plasma phenylalanine levels in 33 control and 23 heterozygous individuals.

ketonuria gene. However, the hereditary nature of the high phenylalanine level in the adult without a family history of phenylketonuria has not yet been demonstrated by a family study.

One individual in the control group had a level of 0.118 μmoles/ml., which was clearly in the range of those heterozygous for the phenylketonuria gene. Unfortunately, this individual would not return for confirmatory tests, and other possible explanations for an elevated phenylalanine level could not be excluded. It is possible, however, that this individual, like the adult included as a presumed heterozygote, was also a heterozygote. The occurrence of two heterozygotes among about 50 control individuals who have been examined so far by either the tolerance test or the basal level would not be unexpected, since the frequency of heterozygotes in the population, calculated from the incidence of phenylketonuria, is between 1 in 75 and 1 in 100 (Knox and Hsia, 1957).

The individual excluded from the mean for the heterozygotes was a 15-year-old sister of a phenylketonuria patient. She was presumed to be a heterozygote because of a typical phenylalanine tolerance test. Her fasting plasma level of phenylalanine was 0.205 μmoles/ml., a value well above all others that were determined and more than six standard deviations above the mean of the levels of other heterozygotes. Her level was substantially below the range of 1.5 to 2.7 μmoles/ml. seen in phenylketonuria (Hsia, Knox and Paine, 1957) and such a diagnosis was not entertained. It is possible that young people have higher levels of phenylalanine than do adults. Adults comprised the majority of the heterozygote group and all of the controls that were studied. The possibility of relatively higher levels in young people is further supported by the results in the two other teen-age siblings of phenylketonuric patients who were presumed heterozygotes and in one additional sibling not previously examined with the phenylalanine tolerance test. The levels in these young individuals (0.136, 0.120 and 0.128 μmoles/ml.) were also in the upper range of all the values found. Other teen-age siblings have been examined whose values were in the normal range.

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## DISCUSSION

Comparison of the results of the present study with those of the phenylalanine tolerance test (Hsia *et al.*, 1956) shows that when about equal sized groups of controls and heterozygotes are studied, nearly the same proportion of individuals will fall into the zone of overlap between the two groups by either test. Neither test will identify with certainty the phenotype of all individuals. However, individuals who cannot be classified with one test may be classified with the other. Three known heterozygotes and two controls whose phenylalanine tolerance curves fell into the zone of overlap between the two groups in the previous study were restudied in the present investigation. Only one of these (a heterozygote) was again in the zone of overlap between the two groups with regard to the fasting level of plasma phenylalanine. The remaining four were clearly classifiable as either controls or heterozygotes by the present test on the basis of levels of 0.043 and 0.073 μmoles/ml. in the two controls and 0.099 and 0.103 μmoles/ml. in the two known heterozygotes. No other individuals who fell in the zone of overlap in the present test had been studied by the phenylalanine tolerance test. These results may indicate that better resolution is possible with the present method, or merely that two criteria are superior to one for the correct classification of an individual. Additional refinements will be necessary before these chemical tests can be used to give an absolute measure of the frequency of the heterozygotes in the population at large.

The overlap between the levels in the two groups may be even less than it appears from the present studies. The zone of overlap is about the same size as the uncertainty of single measurements and as the variation on repetition of the determination at a different time. The overlap may therefore diminish with repetition and increased accuracy of the determinations. The fact that most of the controls were not fasted overnight when studied may have resulted in somewhat increased levels in some individuals in this group, although no general tendency of this sort was noted. The possibility that values slightly elevated above the fasting level might be found in the control group was tolerated since an error in this direction would tend to minimize the difference being sought between the two types of individuals. The major purpose of this study was to establish whether such a difference did exist between controls and heterozygotes and not to develop a test with the highest resolving power.

The control of any factors which cause physiological variations in the level of plasma phenylalanine will be necessary to minimize the instances of incorrect classification. The present data suggest the possible existence of a variation of the phenylalanine level with age. Not enough analyses have been made in the young age groups, however, to confirm this apparent tendency toward high levels in young heterozygous individuals, or to recognize a similar tendency in homozygous normal individuals. No such variation of phenylalanine level with age was noted among the adults in the study. Nor was a sex difference in the levels apparent in either the heterozygote or the control group. No information is available about possible changes in phenylalanine plasma levels with various disease states which might be encountered in a population.

It is not immediately apparent why the individuals heterozygous for the phenyl-

ketonuria gene should have an elevated basal phenylalanine plasma level. Earlier studies (Waelsch, 1955) with a less specific method revealed no difference between the blood phenylalanine levels of controls and heterozygotes. The phenylalanine tolerance test was developed on the assumption that the basal metabolism of phenylalanine in heterozygotes was normal, but that heterozygotes should possess less of the phenylalanine hydroxylase in an effective form than did the normal individuals—an amount adequate for ordinary metabolic purposes but which would be demonstrably deficient in degrading a loading dose of phenylalanine. There was implicit in this reasoning the idea of a metabolic reserve, that is, a fraction of the enzyme activity not usually called upon to function except under high substrate loads. However, it is now apparent that the phenylalanine metabolism in heterozygotes is disturbed to about the same extent both under basal and loading conditions. Both in the fasting state and after administration of 0.1 g. L-phenylalanine per kg., the plasma phenylaline level of the heterozygotes is about 1.5 to 2.5 times that of the normal. It would therefore appear that the enzyme defect caused by a single gene results in the same degree of inefficiency of the reaction at both low and high substrate concentrations. If this is true, the concept of a metabolic reserve must be abandoned in connection with this reaction. The plasma phenylalanine at any level must represent a steady state value determined by the rates of supply from all sources and of removal by all of the effective enzyme present. No pathological consequence of the elevation of phenylalanine level seen in heterozygotes is known, but the persistence of this elevation even under basal conditions increases the likelihood that it may have some physiological effect on the heterozygous individuals.

SUMMARY

The plasma phenylalanine levels were generally lower in thirty-two control individuals (mean = 0.068 μmoles/ml.) than in twenty-two individuals heterozygous for the phenylketonuria gene (mean = 0.103 μmoles/ml.). This test was as sensitive in distinguishing individuals belonging to the two groups as the phenylalanine tolerance test. These findings demonstrate the metabolic effects of a single "recessive" gene, and indicate that the metabolism of individuals with such a gene is disturbed even under basal conditions.

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# Consanguinity in a Midwestern United States Isolate[1]

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ALL MARRIAGES during 1850–1949, of 592 individuals descended from common ancestors J.S. and A.R., were examined genealogically to determine the incidence of consanguineous matings. This couple of Swiss origin immigrated in 1853 to a midwestern farming locale in this country with other members of their family and religious group. These descendants represent a relatively isolated population, the isolating influences being primarily strong religious affirmations and social customs which do not encourage marriage with others outside the religious group.

This study was undertaken after noting 14 cases of an autosomal recessive type of progressive muscular dystrophy (Jackson and Carey) occurring among descendants of the couple J.S. and A.R. (figure 1).

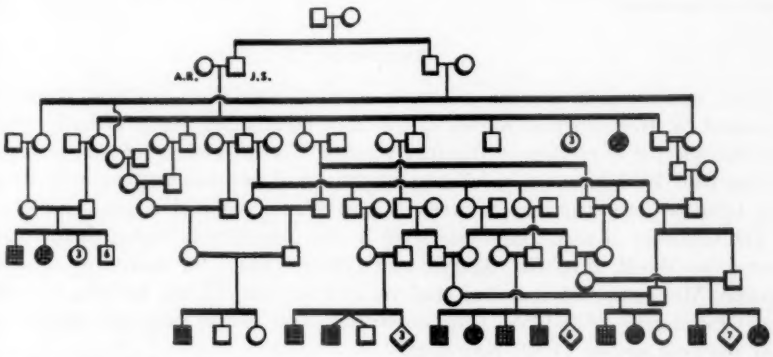


FIG. 1. A portion of the pedigree of descendants of J.S. and A.R. Cross-hatched areas indicate persons affected with muscular dystrophy. Double lined connections indicate a consanguineous marriage.

Data for this investigation were obtained primarily by consulting genealogical records compiled by members of the family and by interrogating certain of the descendants. In several instances, couples were related in two or more different ways. Figure 1 illustrates the complexity of interrelationships encountered. It was necessary to compute the coefficient of relationship of many marriages in order to assign them to the proper cousin category. [The authors wish to express their appreciation to Dr. W. J. Schull, University of Michigan, for his recommendations

Received August 19, 1957.

[1] This investigation was sponsored by the Caylor-Nickel Research Foundation.



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TABLE 1. THE INCIDENCE OF CONSANGUINEOUS MARRIAGES AMONG DESCENDANTS OF J.S. AND A.R. DURING 1850–1949

| Period | No. of Marriages | First Cousin or Closer | Second Cousin up to but Excluding First Cousin | Third Cousin up to but Excluding Second Cousin | Total |
|---|---|---|---|---|---|
| 1850–1874 | 11 | 1 (9.1%) | | | 1 (9.1%) |
| 1875–1899 | 42 | 8 (19.0%) | 3 (7.1%) | | 11 (26.2%) |
| 1900–1924 | 128 | 4 (3.1%) | 34 (26.6%) | | 38 (29.7%) |
| 1925–1949 | 446 | 13 (2.9%) | 72 (16.1%) | 17 (3.8%) | 102 (22.9%) |
| Totals | 627 | 26 (4.1%) | 109 (17.4%) | 17 (2.7%) | 152 (24.3%) |

in establishing exact interrelationships of some of the marriages. The determination of the coefficient of relationship was performed as described in Neel and Schull's text, *Human Heredity*.] Table 1 summarizes the data obtained. The frequencies are slight underestimates, as several probable relationships which could not be verified were not included.

## DISCUSSION

Total consanguinity for this relatively isolated population is almost twice that observed by Herndon and Kerley (1952) in a preliminary study of native, rural families selected at random in Watauga County, North Carolina: of 1651 marriages during 1830–1950, Herndon and Kerley reported 1.57 per cent first cousin marriages and 12.66 per cent marriages between couples related as fourth cousins or closer.

The incidence of first cousin marriages is also considerably higher than that reported by Woolf, Stephens, Mulaik and Gilbert (1956) for marriages occurring between Mormon people and their relatives in Wisconsin, Illinois, Indiana, Michigan and Ohio during 1920–1956. They observed a first cousin marriage incidence of 0.37 per cent among 11,507 marriages.

Because of the Swiss origin of this family, this study was compared with studies of Swiss communities: Brenk (1931), Grob (1934), Ruepp (1935) and Egenter (1934), as quoted by Neel, Kodani, Brewer and Anderson (1949), reported frequencies of marriages of second cousins or closer in various isolated Swiss communities having values of 9.7 per cent, 9.9 per cent, 19.5 per cent and 53.8 per cent respectively, during 1870–1932.

## SUMMARY

All (627) marriages during 1850–1949 of descendants of a couple of Swiss descent who settled in the United States in 1853 were reviewed for consanguinity and 4.1 per cent were found to be between couples related as first cousins or closer and a total of 21.5 per cent between second cousins or closer.

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# Inheritance of The Diego ($Di^a$) Blood-Group Factor[1]

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THE DIEGO BLOOD-GROUP FACTOR ($Di^a$) was discovered in 1953 (Levine and Robinson 1957) and has been studied fairly extensively in the subsequent four years by Arends and Layrisse (1956), Levine, Robinson, Layrisse, Arends, and Sisco (1956), Lewis, Chown, and Kaita (1956), and Junqueira, Wishart, Ottensooser, Pasqualin, Fernandez, and Kalmus (1956). It has a very low frequency in Caucasians and quite a high frequency in American Indians. It is fairly common in those Japanese and Chinese who have been tested, but was not found among a group of 156 Eskimos. Levine et al. (1956, 1957) showed that $Di^a$ is not identical with the other known factors of low incidence (except that Levay was not compared). It was suggested that $Di^a$ is not related to the nine established blood group systems but belongs to a tenth independent blood group system. The evidence for genetic independence of $Di^a$ that has been presented up to the present time, however, is not as complete as one would like. Levine and Robinson (1957) did extensive blood grouping tests in one family in which the factor $Di^a$ occurs, but showed only its probable independence from the Rh and MNS blood groups. They also had suggestive evidence that $Di^a$ and $Jk^a$ are at least not closely related. Since the antibody anti-$Di^a$ reacts only by the indirect Coombs method, it is tempting to think that $Di^a$ may belong in the Kell, Duffy, or Kidd blood group systems, since it is the antibodies of these systems that characteristically react only by the Coombs method.

In 1956 the blood groups of some Quechua Indians in an isolated community of the Peruvian Sierra were tested (Allen and Newman, unpublished). Of 308 tested with anti-$Di^a$*, 74 (24 per cent) were positive, indicating that the gene frequency of $Di^a$ in that population is about 13 per cent. Because the study of the Diego factor was one of the more important aims of the expedition, families were obtained when possible and testing was as complete as possible in the families that were obtained. Table 1 shows the pertinent data on these families. Only four matings of the double back-cross type were found, allowing counts for $Di^a$ vs. P in family 6, $Di^a$ vs. Rh and MN in family 9, $Di^a$ vs. ABO, Rh, MN, and P in family 11, and $Di^a$ vs. Rh and MN in family 18. It can be seen that what little evidence there is suggests no close linkage between the genes for $Di^a$ and those for the ABO, Rh, MN, or P groups. There is some additional evidence of less value in the other families, but there are almost no data on possible linkage with $Fy^a$ (Duffy) and none at all for K (Kell). Unfortunately, the anti-$Jk^a$ (Kidd) serums were not working well, so no reliable

Received October 17, 1957.

[1] The work was supported by Grant No. G2501 from the National Science Foundation and Grant No. G3524 from the National Institutes of Health.

* The anti-$Di^a$ was kindly donated by J. F. Mohn of Buffalo and M. Layrisse of Caracas.

TABLE 1. BLOOD TYPING TESTS OF INDIAN FAMILIES IN SEARCH FOR GENETIC LINKAGES

| | Serial No. | A | B | C | E | c | e | M | N | $Fy^a$ | P | $Le^a$ | $Le^b$ | K | $Lu^a$ | $Di^a$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *BGL family V6* | | | | | | | | | | | | | | | | |
| fa. Marino Reyes Leiva | 114 | 0 | 0 | + | 0 | 0 | + | + | + | + | + | 0 | 0 | 0 | 0 | + |
| mo. Susana Tadeo | 144 | 0 | 0 | + | 0 | 0 | + | + | + | + | 0 | 0 | + | 0 | 0 | 0 |
| Marcela Reyes Tadeo | 145 | 0 | 0 | + | 0 | 0 | + | + | + | + | 0 | 0 | + | | 0 | 0 |
| Leonardo | 146 | 0 | 0 | + | 0 | 0 | + | + | + | + | + | 0 | + | | 0 | 0 |
| *BGL family V9* | | | | | | | | | | | | | | | | |
| fa. Natividad Cilio | 97 | 0 | 0 | 0 | + | + | 0 | + | + | + | + | 0 | + | 0 | 0 | 0 |
| mo. Ciria Tadeo | 178 | 0 | 0 | + | + | + | + | + | + | + | 0 | + | 0 | 0 | 0 | + |
| Maria Cilio Tadeo | 179 | 0 | 0 | + | + | + | + | + | + | + | + | 0 | + | | 0 | 0 |
| Victor | 180 | 0 | 0 | 0 | + | + | 0 | 0 | + | + | 0 | 0 | + | | 0 | 0 |
| Alejandro | 302 | 0 | 0 | 0 | + | + | 0 | 0 | + | + | + | 0 | + | | 0 | 0 |
| Teodora | 479 | 0 | 0 | 0 | + | + | 0 | + | + | + | 0 | 0 | + | 0 | 0 | 0 |
| Juana | 521 | 0 | 0 | + | + | + | + | + | + | + | + | 0 | + | 0 | 0 | 0 |
| *BGL family V10* | | | | | | | | | | | | | | | | |
| fa. Teofilo Leon Huaroma | 176 | 0 | 0 | + | + | + | + | + | 0 | + | 0 | 0 | 0 | 0 | 0 | + |
| mo. Paula Solis Alonzo | 175 | 0 | 0 | + | + | + | + | + | 0 | + | + | 0 | + | 0 | 0 | 0 |
| Manuel Leon Solis | 177 | 0 | 0 | + | + | + | + | + | 0 | 0 | 0 | 0 | + | | 0 | + |
| Marcelina | 164 | 0 | 0 | + | + | + | + | + | 0 | + | 0 | 0 | + | | 0 | 0 |
| *BGL family V11* | | | | | | | | | | | | | | | | |
| fa. Manuel Mendoza Tadeo | 74 | 0 | 0 | + | 0 | 0 | + | + | 0 | + | 0 | 0 | + | 0 | 0 | 0 |
| mo. Maria Lazero Padua | 155 | + | 0 | + | + | + | + | + | + | + | + | 0 | 0 | 0 | 0 | + |
| Rosa Mendoza Lazero | 156 | 0 | 0 | + | + | + | + | + | + | + | 0 | 0 | + | | 0 | + |
| Elena | 157 | + | 0 | + | 0 | 0 | + | + | + | + | 0 | 0 | + | | 0 | + |
| Felix | 158 | + | 0 | + | 0 | 0 | + | + | 0 | + | 0 | + | 0 | | 0 | 0 |
| *BGL family V13* | | | | | | | | | | | | | | | | |
| fa. de la Cruz Mendoza | 199 | 0 | 0 | + | 0 | 0 | + | + | 0 | + | 0 | + | 0 | 0 | 0 | + |
| mo. Corposa Tadeo Cruz | 160 | 0 | 0 | + | 0 | 0 | + | + | + | + | + | 0 | + | 0 | 0 | 0 |
| Margarita Mendoza Tadeo | 161 | 0 | 0 | + | 0 | 0 | + | + | + | + | + | 0 | + | | 0 | 0 |
| Maria | 133 | 0 | 0 | + | 0 | 0 | + | + | 0 | + | + | 0 | + | 0 | | 0 |
| Eusebia | 163 | 0 | 0 | + | 0 | 0 | + | + | + | + | 0 | 0 | + | | 0 | + |
| Francisco | 95 | 0 | 0 | + | 0 | 0 | + | + | 0 | + | 0 | 0 | + | 0 | 0 | 0 |
| Mariano | 134 | 0 | 0 | + | 0 | 0 | + | + | 0 | 0 | 0 | 0 | + | 0 | 0 | 0 |
| Manuel | 74 | 0 | 0 | + | 0 | 0 | + | + | 0 | + | 0 | 0 | + | 0 | 0 | 0 |
| *BGL family V18* | | | | | | | | | | | | | | | | |
| fa. Guillermo Mellizo Meza | 217 | 0 | 0 | + | + | + | + | + | 0 | + | + | 0 | + | 0 | 0 | 0 |
| mo. Nicholasa Guzman Cruz | 216 | 0 | 0 | 0 | + | + | + | + | + | + | + | 0 | + | 0 | 0 | + |
| Fausta Mellizo Guzman | 218 | 0 | 0 | 0 | + | + | 0 | + | + | + | + | 0 | + | | 0 | 0 |
| Eusebia | 219 | 0 | 0 | + | 0 | + | + | + | + | + | + | 0 | + | 0 | 0 | + |
| *BGL family V28* | | | | | | | | | | | | | | | | |
| fa. Nicholas Herrera Cruz | 467 | 0 | 0 | + | 0 | 0 | + | + | 0 | + | 0 | 0 | + | 0 | 0 | + |
| mo. Victoria Isidro Isidro | 198 | 0 | 0 | 0 | + | + | 0 | 0 | + | + | + | 0 | + | 0 | 0 | 0 |
| Provencia Herrera Isidro | 244 | 0 | 0 | + | + | + | + | + | + | + | 0 | 0 | + | | 0 | + |
| Juliana | 243 | 0 | 0 | + | + | + | + | + | + | + | + | 0 | 0 | | 0 | 0 |
| Vicenta | 468 | 0 | 0 | + | + | + | + | + | + | 0 | 0 | 0 | + | 0 | 0 | 0 |
| *BGL family V36* | | | | | | | | | | | | | | | | |
| fa. deceased | | | | | | | | | | | | | | | | |
| mo. Maria Valerio Padua | 271 | 0 | 0 | + | + | + | + | + | + | + | + | 0 | + | 0 | 0 | 0 |
| Andres Copitan Valerio | 491 | 0 | 0 | + | 0 | 0 | + | + | + | + | + | 0 | + | 0 | 0 | + |
| Victor | 352 | 0 | 0 | 0 | + | + | 0 | + | 0 | + | + | 0 | + | 0 | 0 | + |
| Inquiña | 492 | 0 | 0 | + | 0 | 0 | + | + | + | + | + | 0 | + | 0 | 0 | 0 |
| Marcelino | 348 | 0 | 0 | + | 0 | 0 | + | + | 0 | + | + | 0 | + | 0 | 0 | + |

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TABLE 1—*Concluded*

| | Serial No. | A | B | C | E | c | e | M | N | $Fy^a$ | P | $Le^a$ | $Le^b$ | K | $Lu^a$ | $Di^a$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *BGL family V45* | | | | | | | | | | | | | | | | |
| fa. deceased | | | | | | | | | | | | | | | | |
| mo. Asunciana Meza Cruz | 274 | 0 | 0 | 0 | + | + | 0 | + | + | + | 0 | 0 | + | 0 | 0 | + |
| Juandi Everisto Meza | 487 | 0 | 0 | 0 | + | + | 0 | + | 0 | + | 0 | 0 | 0 | 0 | 0 | + |
| Francisca | 245 | 0 | 0 | + | + | + | + | + | + | + | 0 | 0 | + | 0 | 0 | + |
| Margarita | 102 | 0 | 0 | 0 | + | + | 0 | + | + | + | 0 | 0 | 0 | 0 | 0 | + |
| Marsica | 275 | 0 | 0 | + | + | + | + | + | 0 | + | + | 0 | + | 0 | 0 | 0 |
| Teodora | 142 | 0 | 0 | + | + | + | + | + | 0 | + | 0 | 0 | 0 | 0 | 0 | + |
| *BGL family V51* | | | | | | | | | | | | | | | | |
| fa. deceased | | | | | | | | | | | | | | | | |
| mo. Micaela Sanchez Padua | 463 | 0 | 0 | + | 0 | 0 | + | + | 0 | + | + | 0 | + | 0 | 0 | + |
| Cirilo Meza Sanchez | 88 | 0 | 0 | + | + | + | + | + | 0 | + | + | 0 | + | 0 | 0 | 0 |
| Julia | 464 | 0 | 0 | + | + | + | + | + | 0 | + | 0 | 0 | + | 0 | 0 | + |
| Teodora | 465 | 0 | 0 | + | + | + | + | + | 0 | + | + | 0 | + | 0 | 0 | 0 |
| Asuncion | 466 | 0 | 0 | + | + | + | + | + | 0 | + | 0 | 0 | + | 0 | 0 | 0 |
| *BGL family V55* | | | | | | | | | | | | | | | | |
| fa. Gregorio Vega 2º Leyva | 455 | 0 | 0 | + | + | + | + | + | 0 | + | + | 0 | + | 0 | 0 | + |
| mo. deceased | | | | | | | | | | | | | | | | |
| Margarita Vega Moran | 295 | 0 | 0 | + | 0 | 0 | + | + | + | + | 0 | 0 | 0 | 0 | 0 | 0 |
| Marcelina | 456 | 0 | 0 | + | + | + | + | + | 0 | + | + | 0 | 0 | 0 | 0 | 0 |

All were D-positive, $C^w$-negative.

TABLE 2. 2 × 2 CORRELATION TABLES, QUECHUA INDIAN DATA

| | A+ | A− | C+ | C− | N+ | N− | Fy(a+) | Fy(a−) | P+ | P− | Le(b+) | Le(b−) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Di(a+) | 4 | 70 | 51 | 23 | 44 | 30 | 55 | 10 | 29 | 45 | 50 | 23 |
| Di(a−) | 17 | 217 | 184 | 50 | 132 | 95 | 174 | 16 | 109 | 118 | 162 | 53 |
| $\chi^2$ | 0.3 | | 3.0 | | 0.04 | | 2.6 | | 1.7 | | 1.3 | |

| | E+ | E− | c+ | c− | e+ | e− | | e+ | e− |
|---|---|---|---|---|---|---|---|---|---|
| C+ | 136 | 99 | 143 | 92 | 232 | 3 | c+ | 148 | 68 |
| C− | 73 | 0 | 73 | 0 | 8 | 65 | c− | 92 | 0 |
| $\chi^2$ | 45.7 | | 40.8 | | 250 | | $\chi^2$ | 37.3 | |

| | c+ | c− | e+ | e− | | N+ | N− |
|---|---|---|---|---|---|---|---|
| E+ | 204 | 5 | 141 | 68 | M+ | 141 | 125 |
| E− | 12 | 87 | 99 | 0 | M− | 35 | 0 |
| $\chi^2$ | 230 | | 41.2 | | $\chi^2$ | 28.1 | |

*Footnote:* Chi square test shows no relation between $Di^a$ and the other factors. The high correlations among the Rh factors and between M and N are given for comparisons. Absence of correlation does not prove the absence of genetic relation, however.

data were obtained on the Kidd groups. Both Amerindian and Caucasian populations are difficult for study of the possible relation of $Di^a$ to $Fy^a$ and K, because Di(a+) is so rare in the latter and K+ and Fy(a−) are so rare in the former. The best population to test would be the Mestizo.

In table 2 are presented 2 × 2 correlation tables which indicate no serologic relation between $Di^a$ and the ABO, Rh, MN, Duffy, P, or Lewis systems, and probably no close genetic relation of the sort that exists between C and E of the Rh system or M and S of the MNS system.

Finally, 45 Di(a+) Quechua bloods were tested with anti-$Kp^a$ of the Kell system (Allen and Lewis, 1957). All were negative, showing that $Di^a$ and $Kp^a$ (which is uncommon in Caucasian populations) are not the same. Since none of the Indians was Kp(a+), no correlations with $Di^a$ could be made. Also, one individual carrying the rare Kell gene $K^o$ (Chown, Lewis and Kaita, 1957, Allen, Lewis and Fudenberg, unpublished) has been shown negative with anti-$Di^a$.

## DISCUSSION

The family studies shown in table 1 give no indication of illegitimacy, so the linkage counts are presumably reliable. The data were quite unrevealing, however, due partly to the unfortunate blood group frequencies of the population tested, and partly to bad luck.

Considering these data along with previous reports, there is now fair evidence that $Di^a$ is not related to the Rh or MN groups. There is some evidence that it is not related to the ABO, P, Lewis, Duffy, or Kidd systems. There are no data for or against a relation to the Kell or Lutheran groups. If $Di^a$ is related to either of these, it would seem more likely to be related to the Kell groups because of the similarity of reactivity of the antibodies.

## SUMMARY

Data regarding the inheritance of the blood-group factor $Di^a$ (Diego) were obtained through the testing of over 300 Quechua Indians in Peru, including a number of families. Evidence is still incomplete regarding the possible relation of $Di^a$ to the blood group factors of the nine established systems.

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# Studies in The Human Sex Ratio

## 5. A Genetic Explanation of the Wartime Increase in the Secondary Sex Ratio

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ONE OF THE most controversial subjects in the study of the human sex ratio is the assertion that there is a marked rise in the proportion of male births toward the end and immediately after a major war. Even those investigators who seem to have proven the existence of an increase do not agree on its causes. Most of the European investigators such as Savorgnan (1921) and Marbe (1940) are agreed that, at least for England, France, and Germany, the increase is statistically significant and have offered one or more of the following explanations: The proportion of first births is increased in wartime; The average age of the mother is lower; There are fewer miscarriages; The interval between births is greater; etc.

All of the above mentioned explanations were disproven statistically in two recent papers (Ludwig and Boost, 1943, MacMahon and Pugh, 1954). MacMahon and Pugh also have refuted the claim made by some biometricians that the secondary sex ratio did not change significantly in the U. S. A. during either World War I or II. Such erroneous conclusions are arrived at, as these writers have shown, when one works with birth registration data of the *whole* U. S. A. which, for the pre-World War II years were incomplete and unreliable in many states.

When MacMahon and Pugh examined birth data from only those states in which 98 per cent or more of white live births are known to have been reported (R. I., N. J., Mass., N. Y., and Conn.), a statistically significant rise in the secondary sex ratio was found for the years 1945 to 1947 inclusive, the peak occurring in 1946.

Neither Ludwig and Boost, nor MacMahon and Pugh offer explanations for the wartime sex ratio rise. In 1948, this author published a paper in which she gave some indication of how an alternative explanation could be developed. She demonstrated on 880 individuals in "Wer Ists?" the German "Who's Who", with at least one child born during World War I and another born either before or after the war, that the wartime rise does not occur *within* individual families. The sex ratio for 1,080 children born between 1915 and 1918 inclusive was about half-way between that of their siblings born either before or after the war. On the other hand, the sex ratios for both the pre-war and post-war periods were higher in these 880 families than in another group also listed in "Wer Ists?" married during the same interval but with no offspring born during the war. In 1948 the author stated that she believed that at least one cause of the wartime rise in the sex ratio might be a proportional increase in births from families with a higher incidence of male births at any time.

Received November 2, 1956.

The statisticians of the Metropolitan Life Insurance Co. (1949), though not believing in a general wartime rise, had stated that "during the war period there appears to have been some increase in the sex ratio of total births among white mothers under 25 years of age". Assume that wives of soldiers tend to be less than 25 years old, and are separated from their husbands for long periods of time with diminished opportunity for fertilization. Doubtless, some couples conceive more easily than others. If such differences are associated with differences in the primary sex ratio then, in wartime there should be a shift in the sex ratio in the direction of that for offspring from families which conceive more easily.

The tenth edition of "Wer Ists?" lists date of marriage and birth date of children for each of its entrants which makes it possible to determine the time interval between onset of marriage and birth of first child. Of course, the question arises whether illegitimacy and birth control could have affected these data to any appreciable amount. The author is inclined to rule out illegitimacy entirely for families listed in the German "Who's Who". The largest single group listed is the higher aristocracy, mostly members of the princely houses which ruled Germany until 1918. They married almost only among themselves and both weddings and births were events accompanied by much publicity; premarital relations of male members were confined to the servant class. Another large group are professors and other university graduates with M.D., Ph.D., or L.L.D. degrees, their wives being chosen almost always from the upper middle class (occupation of father-in-law is also listed) and large dowries were the rule, at least until the inflation. Here also premarital relations of the males, who usually did not marry until they had established themselves in their profession, were confined to the servant class. The author does not believe that in these classes birth control before a first or second child was much in practice. The ruling classes wanted heirs and the upper middle class desired at least one or two children and were financially able to provide for them.

A sample of 2,000 families in which the parents were married between 1900 and 1919 was drawn and divided into two groups: (a) a more quickly fertile group in which the first child was born within the first 18 months after date of marriage, and (b) a more slowly fertile group in which the first child was born more than 18 months after date of marriage. We chose 18 months as time of division because the sex ratio, rising towards the end of the war, remained high for about 18 months after demobilization, though by trial and error it seemed that the choice of 15 months as time of division might have given more significant results. Since parity is known to have considerable influence on the sex ratio, I computed the ratio for first born only.

## RESULTS

In group (a) 55.3 per cent of a total of 1,178 children were sons, whereas in group (b) only 49.8 per cent of 822 children were sons. The difference of 5.5 per cent was 2.4 times its standard error and statistically significant at the two per cent level.

## CONCLUSIONS

In wartime, when opportunity for fertilization is decreased in a large segment of the population because husbands are away in the armed forces except for short leaves,

proportionally more children will be born to the "more quickly fertile" parents and, since "more quickly fertile" parents apparently have more than the average number of sons, the overall sex ratio will be raised. Of course, another cause for the difference in secondary sex ratio between the two groups differing in ease of reproduction may be differences in the frequency of abortions. Abortions are known to be more likely males than females and, since an abortion will delay the event of a live birth within a single family, abortions would tend to decrease the sex ratio of the more slowly fertile group; i.e., abortions might be one cause of the existence of the more slowly fertile group. Another cause may be differences in strength of sexual drive, a theory based on a related finding in the animal world. Dr. E. W. Rasmussen of the Genetics Institute of the University of Oslo, Norway, supplied us with a small set of birth statistics on two strains of albino rats with sexual drives of differing strength. For rats with strong sexual drive the proportion of male offspring was found to be 54.6 per cent as against only 48.2 per cent for rats with weak sexual drive. Because of the small number of cases (314) the difference is statistically not reliable, but the result is in the direction anticipated and gives some indication of a possible cause for differences in speed of fertility observed in the present set of data.

The above results may be considered further support for the theory advanced by the author (1951 and 1954) that in man we have the same lines for high and low sex ratio obtained by King (1918) in selection experiments on rats. This theory recently found added support in an anthropological study by Martinez-Gustin (1956) who found a strong relationship between the somatotype of parents (expressed by Rohrer's index for both father and mother and combined into a common ratio M) and the sex ratio of offspring. This relationship between M and the sex ratio of the children was particularly strong in the unisexual sibships of Martinez's data.

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# BOOK REVIEWS

## *Experimental Designs*

By William G. Cochran and Gertrude M. Cox. New York: John Wiley and Sons, 1957. Second ed. xiv + 611 pages, and five unnumbered pages of tables. $10.25.

This book "... is directed at the experimenter and is intended to serve as a handbook which is consulted when a new experiment is under consideration." The first edition was immediately recognized as an outstanding contribution, and this second edition, including about 160 additional pages, will be similarly received.

Of general interest to workers in human genetics will be the first three chapters, dealing briefly with the philosophy of experimentation (under the headings "The contribution of statistics to experimentation," "Statistical inference," and "The function of randomization") and the generalities of planning, and devoting a chapter to "Methods for increasing the accuracy of experiments" and one to "Notes on the statistical analysis of the results" (based on the assumption "... that the reader has some knowledge of the principles of analysis of variance and of the computational methods involved").

Of the remaining fourteen chapters, twelve take up in considerable detail various types of design, including many worked examples, one discusses the "Analysis of the results of a series of experiments," and one discusses the technique of randomization and gives tables of random permutations of 9 and of 16. The chief additions in the second edition are Chap. ter 6A, on fractional replication, and Chapter 8A on methods of studying response surfaces. Tables of significance levels of the t and F distributions have also been added.

This book has already proved its value to research workers. Though human genetics is largely nonexperimental and will necessarily remain so, opportunities for profitable use of this book will occur wherever the work becomes experimental (perhaps chiefly in the development of methods), in serology, organoleptic testing, and the like.

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## *An Introduction to Probability and Its Applications*

By William Feller. Vol. 1, second ed., New York: John Wiley and Sons Inc. 1957.

The first edition of this book was published in 1950, with the expressed purpose of combining a rigorous mathematical treatment of probability theory with a more informal introduction to practical applications. The success of this approach has been so great as to require a second edition, revised by addition of new material and correction of some minor errors. The elementary chapters have been expanded, and the topic of waiting times has been introduced in an early section. The result is a book that can be studied with profit by the non-mathematician interested primarily in applications, or by readers at an advanced level of mathematical sophistication. The exposition is clear and unified, and proofs and applications alike are given with a remarkable simplicity and conciseness. There appear to

be few misprints in the new edition, the most conspicuous being the omission of an exponent in the definition of the normal density function on page 164.

The introduction and first chapter present the basic definitions of probability in the context of discrete sample spaces, with enough examples to make the student appreciate the intuitive appeal and elegance of this formulation. Then follows an introduction to combinatorial analysis, occupancy problems, and conditional probability. Geneticists will appreciate the inclusion of translocations as a problem in combinatorial analysis and of a number of topics in population genetics in the chapter on conditional probability. Classical results are obtained with notable ease, and the utility of stochastic matrices in studying the distribution of relatives is illustrated in an informal and appealing way. This early introduction of stochastic matrices is a prelude to an intensive consideration in connection with random walks and Markov chains. Other early chapters treat the binomial and Poisson distributions, the central limit theorem, random variables, and generating functions. The last part of the book is devoted to Markov chains and other stochastic processes, the analytical treatment of which has been such a powerful method in population genetics.

Human geneticists will find this a most comprehensive and comprehensible book on probability theory. Nearly all problems in human genetics require probabilistic methods for their solution. It is at once humbling to reflect how little we have made use of these elegant tools, and exhilarating to consider the armamentarium at our disposal in this volume.

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### *An Introduction to Genetic Statistics*

BY OSCAR KEMPTHORNE. New York: John Wiley, 1957. pp. 545, $12.75.

PROFESSOR KEMPTHORNE frankly states in Preface: "The present book is not a book on breeding theory and procedures, and defers to the famous book *Animal Breeding Plans* of J. L. Lush on all such matters." Apparently unable to appreciate the wisdom of the author, the publisher lists "the theory of animal and plant breeding" in bold type in a full-page back-cover advertisement as one of the "important topics covered" in the book! The wise decision to exclude plant and animal breeding practice, however, should not be taken to mean that the book will not be useful to animal breeders. Quite the contrary, this fine collection of statistical methods will prove very useful to many animal breeders. The statistical investigation of evolutionary theories is not considered. This omission is also wise.

All authors, in science or any other field, have personal preferences, tastes, and interests in certain aspects of a subject. This is what makes authors differ. I do not advocate the avoidance of personal taste. Indeed, I do not think personal taste can be avoided. In particular, every author likes to use the tools of which he is a master. Therefore, we respect Kempthorne's preference for long algebraic proofs as evidenced in this book, but by the same token we expect him to tolerate approaches other than his own. Scattered here and there throughout the book are remarks which lead me to suspect that the author may lack historical perspective of scientific developments (except for Fisher's work). An original contribution made forty years ago cannot be expected to have the same generality as one which has been worked out in the Spring of 1957. Later improvements are expected of the younger scientists, whose results will, in turn, be improved by others. There is no point in criticizing certain old results because they are based on restricted conditions. All results are. It is our responsibility to improve them.

This book really consists of three books: (I) on general statistical methods; (II) on clas-

sical (Mendelian) population genetics; and (III) on the analysis of quantitative characters usually known as biometrical genetics (the divisions are mine—C. C. Li). Each of these three parts may be read more or less independently. The first chapter gives the normal distribution function (and other elementary topics) which is repeated on page 217, but nowhere in the rest of the book does the author make use of that expression explicitly. The moment generating function (page 22) is another example of a method being presented but not used. Many others may be found throughout the book. Some of the chapters require no knowledge of genetics whatsoever. The author realizes all this, for he says at the opening of Chapter 15 which is the beginning of Book III (name given by me) on quantitative genetics: "The previous chapters have provided us with the tools . . . which have utility in problems quite distinct from genetic ones." In other words, they are general statistical methods. Of the three, Book III is by far the most important. Certain other topics are merely auxiliary. A conspicuous example is Chapter 10 entitled "Statistical problems in human genetics." Actually, it deals with the single problem of the segregation of Aa x Aa families and nothing else. "A statistical problem in human segregation" would be a more appropriate title.

The book contains a great wealth of material, a considerable proportion of which concerns recent developments and contributions of the author and his former associates. The rewriting of certain old results is usually well done. For example (I say this is an example; there are many other similar cases), the "direct method" (pages 325–328) of demonstrating that the covariance (parent, offspring) $= \frac{1}{2}\sigma_A^2$ and Cov. (full sibs) $= \frac{1}{2}\sigma_A^2 + \frac{1}{4}\sigma_D^2$ is refreshing and elegant, as well as lucid.

It is a pity that with so much valuable material at his disposal, the author does not seem to have paid enough attention to the organizational aspect of the book. If all the sections of all chapters were reassembled according to some more logical sequence, the book would take on a new look and would be much less bulky.

Apart from the organization, the technique of presenting certain results is not always orderly. For instance, in the case of a four-rank hierarchical classification, there are only five basic quantities to be calculated, viz., in increasing order of, say, 1, 2, 3, 4, 5:

$$\frac{Y^2}{N}, \quad \sum_i \frac{Y_i^2}{N_i}, \quad \sum_{ij} \frac{Y_{ij}^2}{N_{ij}}, \quad \sum_{ijk} \frac{Y_{ijk}^2}{n_{ijk}}, \quad \sum_{ijkl} y_{ijkl}^2$$

so that the difference between any two adjacent quantities is a required sum of squares in the analysis of variance. Expectations should be given in the same order so that the difference between any two adjacent expectations is the required expectation of the corresponding sum of squares. The results take a naturally systematic form. But the author (pages 240–242) chooses to do it in the order of 1, 4, 3, 2, 5, without any apparent advantage.

In the above expressions I have omitted the dots in the subscripts of the original notation because they are unnecessary for hierarchical classifications. The author himself noted that $Y_{.j.}$ is meaningless in such cases (page 236). But, by and large, the author is not consistent or careful about notation. There are too many lapses to enumerate and a few examples will have to suffice: The $ND$ of page 146 is the $x$ of page 148. The $P_i$ and $P_j$ in Chapter 9 are unnecessarily confusing. The $p$ of page 192 has an entirely different meaning from the $p$ of the opposite page. On page 193, $s$ is the number of recessive members in a sibship of size $k$; on the next page, however, $r$ is the number of recessives and $s$ has completely disappeared. Then, on the next page (page 195 now), we encounter "a family of $k$ offspring with $s$ recessives" again! The capital letters of $y = \mu + G + E$ on page 224 become lower case letters $g$ and $e$ on the very next page. The summation signs $\sum_{ijk}$ and $\sum_{ij}\sum_k$ appear on the same page (241). Probably the worst example is $V(x_{1.3}^2)\ V(x_{2.3}^2)$ at the top of

page 275. We can either use $V(x)$ to mean the variance of $x$, or $E(x^2)$ to mean the expectation of $x^2$, but certainly not $V(x^2)$.

In the section dealing with path coefficients, the author finds regression coefficients "of more value" (page 293), because they "tell us . . . about the structural relationship between the variables." Even a limited discussion of this subject would take several pages and I do not propose to do it here. Only three very brief remarks should suffice: (i) All the physical units used are entirely arbitrary. Just how these arbitrary numbers could make a real contribution to our understanding of the relationship of variables is not clear to me. (ii) A distinction between statistical methods and the knowledge of a subject matter should always be kept in mind. The formulation of a relationship is based on the knowledge, working hypothesis, or the particular purpose of the investigator, while a technique is employed to describe the formulated structure. (iii) There is no point in arguing whether this or that is of more value It depends so much on the actual situation under consideration and the purpose of the investigator.

Regarding the "causal diagrams" which usually go hand-in-hand with the method of path coefficients, the author commented (page 288): "Actually in this case as in all the other cases discussed in the literature, the path diagrams appear unnecessary except insofar as they provide a visual picture of the causal forces in the situation. The actual equations in standardized variates seem to be easier to manipulate and lay bare the quantitative nature of the causal relationships assumed." Of course, this statement is correct. In this sense, the table of the analysis of variance, of which there are dozens and dozens in this book, is even less necessary for analytical work. And so are the ordinary graphs showing how one variable changes with another, because the equation $y = f(x)$ has completely defined the quantitative nature of the relationship. A path diagram, being a visual aid of precise formulation, does more than a graph or a table. Furthermore, one never "manipulates" a diagram; one also works with equations in an analysis based on path coefficients. While we are on this subject, we may well note that the author's diagram for double first cousins (Fig. 15.7, page 335) is hardly an improvement over the conventional form. One may also wonder what useful purpose Fig. 17.1 (page 370) serves. It is misleading while the verbal description is clear.

In the final chapter of the book, the author has devoted only 8½ pages to "selection for quantitative characters."

Most of the misprints are quite obvious and need not cause confusion. Perhaps a more serious one occurs in the bottom line of page 160 where the numerator of the $\chi^2$ component for $F_2$ data is given as $(n_1 + n_3 - 2n_2)^2$ when $(n_1 + n_3 - n_2)^2$ is meant.

Several misprints or minor slips on the part of the author occurred in the section on path coefficients. The coefficient of determination should be $d_{x_i \cdot x_o}$, not $d^2_{x_i \cdot x_o}$ (page 287). In Fig. 14.8 (page 296), the line for $r_{15}$ has only one arrowhead and is in the wrong place. In Fig. 14.9 (page 298), the arrowheads for correlations are missing altogether. (Incidentally, I think this is possibly an improvment in notation, because a line without arrowheads can be taken to be a path that may be used in either direction, just as a street without a one-way sign is understood to be a two-way street.) In Fig. 14.10 (page 301), the double headed line for the correlation between the full sibs $H_3$ and $H_4$ is redundant and should not be there at all; or, if the author so wishes, a dotted line may be employed.

The bibliography at the end of each chapter is very good—extensive and up-to-date. An examination of only a few of the listed papers will carry the reader far into the subject. It should be noted, however, that some of the references actually have very little to do with the subject matter of the chapter to which they are appended; they are listed there presumably to broaden the student's interest.

The index, 29 pages long, is complete, and should be very useful for those who wish to read certain selected topics. In conclusion I must say, despite my criticisms, of some minor points, that this is an extraordinarily good book and my fellow biologists should be as much benefited by reading it and as grateful to Professor Kempthorne as I am. Never have so much statistics and genetics been brought together in one volume.

A final personal remark: The second sentence in the preface is "The author is not a geneticist," but I insist that he is one of our best. Still we may be unfair in asking him what he means by the "transmission of genotypes and of qualitative phenotypic characters" (page 78).

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***Biochemistry of Some Peptide and Steroid Antibiotics***

By E. P. Abraham. New York: John Wiley & Sons, Inc. 1957. pp 96, $3.00.

This little book outlines the step-by-step procedures used to prove the structure of several antibiotics: Bacitracin A, Cephalosporins P, N and C. It attempts to show that a certain unity exists between antibiotics that superficially differ quite widely. The majority of the book is concerned with organic chemistry of the antibiotics in question. The last section presents some data and speculation on the biogenesis and mode of action of these antibiotics. The development of bacterial resistance to penicillin is mentioned, and the possible clinical application of Cephalosporin C in the treatment of infections resistant to penicillin is discussed. This work may have certain implications in genetics. The author is Senior Research Officer at the Sir William Dunn School of Pathology, Oxford University, and has been active in the field of antibiotics since the early days of penicillin.

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***The Aleut Dentition: A Correlative Study of Dental Characteristics in an Eskimoid People.***

By Coenraad F. A. Moorrees. Cambridge: Harvard University Press. 1957. pp. 196 + x $4.50.

Moorrees' monograph is one of a number of publications resulting from an expedition to the Aleutian Islands chain sponsored by the Peabody Museum of Harvard University in 1948. It was found that less than 1,200 Aleuts remain alive today, "over half of whom reside outside their aboriginal homeland." The Peabody expedition discovered a total of 156 Aleuts living in two villages, one on Umnak Island, the other on Atka Island. Laughlin has distinguished between Eastern and Western Aleuts, claiming each a breeding isolate. He based his hypothesis on anthropometric, serologic, ethnologic and linguistic differences between the two groups noted in 1948. Laughlin's hypothesis that the Eastern and Western Aleuts constitute not one but two breeding isolates is the basic motivation for Moorrees' study of the Aleut dentition.

There are a number of goals which Moorrees set for himself in this study: (1) to determine whether the dental traits previously found in Mongoloid populations apply to the Aleuts; (2) to use the dentition to verify the presumed differences between two closely

genetically related Aleut populations; (3) to differentiate between Eastern and Western Aleuts by utilizing the dentition; (4) to evaluate differences in development patterns between Aleuts and Whites by using data on tooth emergence; (5) to offer some information on the genetic origin and mode of inheritance of a type of malocclusion prevalent in the Aleuts; (6) to evaluate the effect of environment on the incidence of dental disease, because within the last 50 years striking environmental changes in mode of living, diet and health status of the Aleuts have taken place while "the genetic factors have been much more stable during this period."

The book consists of nine chapters. There is a brief introductory account of the environment, history and physical anthropology of the Aleuts, followed by a chapter outlining the objectives of a dental study in populations of different racial stocks. Chapter 3 describes the nature of the data and the statistical treatment. A total of 52 male and 42 female inhabitants possessing a permanent dentition were examined. Since this represents slightly over 60 per cent of the total number of inhabitants living on the Aleutians today, and represents a considerably greater proportion of those possessing permanent dentitions. Chapter 4 presents pertinent data regarding the diet of the Aleuts. We can only wish that more of this type of information was included in the population studies by anthropologists, epidemiologists and demographers. The fifth chapter presents a description of the morphological characteristics of the dentition found among the Aleuts. These include: shovel shaped incisors, cusp numbers of mandibular second premolars and maxillary molars, Carabelli's cusp, cusp numbers and group patterns of mandibular molars, taurodontism supernumerary cusps, supernumerary and missing teeth. In addition, torusmandibularis and gingival pigmentation are described. Moorrees proceeds with his analysis of the morphological aspects of the dentition using a modification of Dahlberg's interpretation of Butler's "field concept" and dropping the terms "primary" and "secondary" traits. He describes the dentition in terms of "intensification", "simplification" or "retention" of basic characters. Examples of intensifications are: marked shovel shaped incisors, large cusps of Carabelli, hypotaurodontism and marked mandibular tori. Simplifications would be: the "plus" groove patterns in mandibular molars, reduction in the number of cusps of mandibular second premolars, maxillary and mandibular molars, and reduction in the number of teeth. Moorrees interprets the relatively greater prevalence of four cusps in the maxillary second molar and five cusps in the mandibular second and third molars as examples of retention of basic traits. We are happy to observe, however, that Moorrees speaks of these basic traits as "the hypothetical basic human pattern." Moorrees finds that the Aleut dentitions have departed less from this hypothetical pattern than have the dentitions of other racial groups. Similar findings have been made for the American Indians and for the East Greenland Eskimos. Within the Aleuts themselves, however, the Eastern Aleuts, the more recent immigrants, exhibit more "intensification" of basic characteristics than does the Western group, although in two traits the Eastern Aleuts show a "simplification in their morphology." Moorrees claims that these inter-population differences in the dentition strengthen Laughlin's hypothesis that the population of the Aleutian "consists of two different breeding isolates." This seems to the reviewer to be putting the cart before the horse. Evidence of isolation is more satisfactorily gained from an examination of marriage records. Once the fact of breeding isolation, or rather relative breeding isolation, is determined, then let the morphological chips fall where they may. The assumption of the hypothesis of "basic" patterns in the dentition and of the implicit understandings in the terms "intensification" and "simplification" lead Moorrees to a cul-de-sac. He is unable to find any satisfactory explanation of why these "trends" exist. He is forced to conclude that the dentitions do not offer sufficient data to explain why different trends toward intensification or retention of basic characters exist in

the dentitions of Eastern and Western Aleuts. He finds three possible explanations: (1) admixture with Whites, (2) the survival of Paleo-Aleut traits, and (3) admixture with people from the second Eskimoid invasion. We might well ask the question, "on what grounds has genetic drift been eliminated from consideration as a primary factor in these different frequencies, particularly in view of the small size of the populations involved?"

The sixth chapter, labeled Odontometry, contains a number of tables presenting measurements of the various teeth and aspects of the teeth. It is accompanied by an appendix giving comparative odontometric data of different populations. These findings, too, according to Moorrees, concur with the implications of the field concept hypothesis. Chapter 7 deals with tooth position, occlusion, and dental disease. Because of the small number of children involved, no conclusions could be drawn regarding tooth emergence among the Aleuts. The bulk of the chapter is devoted to a review of others' findings on this subject. Using Angle's classification to describe Aleut malocclusion, Moorrees finds that, as with Whites, the most common Aleut malocclusion is of the Class I type. However, the Aleuts differ in a striking way from Whites in showing a relatively high prevalence of Class III malocclusion and no cases of Class II malocclusion! Fourteen cases out of 107 exhibited Class III malocclusion. All of these cases were found in Aleuts who belonged themselves, or who had one or both parents belonging, to the Eastern group. Moorrees is very cautious in accepting the suggestions that mandibular prognathism is inherited according to simple Mendelian dominance or recessiveness, although he is perhaps correct in suggesting a hereditary basis for its genesis.

Space will not permit consideration of the many other aspects of the dentition of the Aleuts which Moorrees has included in his book. We commend, on the whole, his cautious attitude in accepting "pat" genetic explanations for the different frequencies of various morphological traits, even though this reluctance leads perhaps to the feeling that the work has contributed little to an overall understanding of the problems he poses in the introduction. To the reviewer at least, the placing of the 41 figures of graphs, tables and illustrations in front of the textual material dealing with them, is a little puzzling and somewhat disconcerting. We would have preferred their inclusion at points where references were made to them. We feel that perhaps a more respectable framework could have been found for this important work if the central theme had not been so slavishly devoted to an exposition of the hypothesis of breeding isolates. Beyond question this work by a capable and conservative odontologist deserves ranking with the more important studies of populational dentition. The fact that Pederson's work on the Greenland Eskimo and Moorrees' present work on the Aleuts are both devoted to an Eskimoid people, emphasizes the great need for similar studies on other racial groups. Moorrees' own work is hampered by the fact that there is so little comparative data to work with. It is to be hoped that future studies will take advantage of Moorrees' careful observations and methods in making available to geneticists and physical anthropologists alike more extensive comparative data. Perhaps then some of the genetical and racial answers to the questions that Moorrees raises can be provided.

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# LETTERS TO THE EDITOR

## Y-Linkage in Man

November 19, 1957

To the Editor
Dear Sir:

In my paper on "The problem of complete Y-linkage in Man" (*Am. J. Human Genet.* 9: 147–166, 1957) I have tried to summarize information on the genetics of the Y-chromosome in organisms other than Man. Having overlooked important studies on the fly *Aphiochaeta xanthina*, I should like to refer to them herewith. In a series of papers (1949–1955) published in Japanese, Chiyoko Tokunaga described partial sex-linkage of several genes and proposed a hypothesis concerning the role of the Y-chromosome in sex determination of this species. A review paper, in English, appeared in 1955: Tokunaga, C., The presence of male determining factors in *Aphiochaeta xanthina* Speiser. Kobe College Studies 2: 1–32. An independent account of partial sex-linkage in Aphiochaeta was published by Ondraschek, H., 1953, Vererbungsstudien an *Aphiochaeta xanthina* Speis. (Phoridae). Z. I. A. V. 85: 347–353.

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## Rh Terminology

November 22, 1957

To the Editor
Dear Sir:

Boyd's review of the Spanish edition of Wiener's Rh-Hr Syllabus may mislead those unfamiliar with the subject. Since C-D-E protagonists have been unable to disprove *what* Wiener has written in criticism of their publications, they make an issue of *how* he writes, and now Boyd finds fault with the *quantity* he has written on the question. Those desiring to learn the fundamentals of the subject should not be deterred from reading the Rh-Hr Syllabus by Boyd's review, but should study all the material available on the subject—by C-D-E workers as well as by Wiener—so that they can draw their own conclusions. The debate concerns not merely a difference in opinion and of nomenclature, but also deals with the accuracy of important serological observations and the correctness of certain fundamental scientific facts. Boyd asserts that the reader of the Rh-Hr Syllabus will learn nothing of the British viewpoint even though he admits that as much as 25 per cent of the volume is devoted to a discussion of C-D-E, but at the same time he fails to explain how readers are expected to learn about the important difference between a blood factor and an agglutinogen or the theory of multiple alleles from British books on blood grouping, which devote no or hardly any space to these important subjects.

There is no doubt that no argument can be advanced either against the Rh-Hr theory or in favor of the C-D-E hypothesis which cannot easily be refuted. For example, at a recent meeting of the American Association of Blood Banks Wiener presented a paper on Rh Nomenclature before a large audience including leading C-D-E sponsors. The latter were requested to answer any of the eight questions recently published in The Lancet (Wiener, A. S.: The Rh Blood Groups. The Lancet, April 13, 1957, pp. 791–792), in which are pointed out fallacies in the C-D-E philosophy, or to pose any questions they wanted answered. It is significant that the leaders of the C-D-E philosophy who were present failed to avail themselves of this opportunity.

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